Planktonic Archaeal Diversity and Ammonia-Oxidizer Abundance Change with Depth in Lakes Malawi, Kivu and Superior

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Juana Muñoz Ucros

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Abstract

Planktonic *Archaea* may play a key role in the nitrogen cycle by oxidizing ammonia, but little is known about these microbes in large lakes of the world. Differences in the abundance of total *Archaea*, marine group I *Archaea* (MG-1), and ammonia-oxidizing *Archaea* (AOA) measured in 2010, 2011, and 2012 were compared during stratified conditions in Lake Malawi, a tropical African great lake, to previous work completed in Lake Superior and Lake Kivu. Total *Archaea*, MG-1, and AOA abundances increased by more than two orders of magnitude in Lake Malawi from the warm epilimnion to the oxic upper hypolimnion during thermally stratified conditions from November to January, but remained abundant in the deeper anoxic hypolimnion. 16S rRNA clones related to the Thaumarchaeota, possible ammonia oxidizers, and archaeal clones from previous work in Lake Victoria were present in both Lake Malawi and Kivu, and euryarchaeal clones were common in the deeper anoxic waters. The distribution and diversity of planktonic *Archaea* in this tropical great lake was similar to that in Lake Superior, a temperate great lake of comparable trophic status.

While more detailed seasonal work about archaeal abundance and community diversty has been completed in the western basin of Lake Superior, spatial patterns of archaeal distribution have not been evaluated across this lake. Here, I compared the abundance of these 3 archaeal gene markers in the epilimnion and hypolimnion from seven sites across Lake Superior during the stratified period in 2009 and 2010. The abundance of the total *Archaea*, MG-1, and AOA was consistently lower in the epilimnion, and at least an order of magnitude higher in the hypolimnion at all sites. Although my aim was not to elucidate the causes of such distribution in Lake Superior, this study does provide additional evidence that planktonic Archaea are more abundant in colder waters of the hypolimnion and their abundance is restricted in the surface waters of thermally stratified lakes.

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CHAPTER I

Introduction

It is widely accepted that microbial metabolism sustains all life on Earth, catalyzing the most essential reactions in all geochemical and nutrient cycles (Cortner and Biddanda, 2002; Schleper et al., 2005; Jarrell et al., 2011). Carbon and nitrogen cycling are especially dependent on the activity of microorganisms in inland water ecosystems, from rivers, streams and ground water to large lakes (Briée et al., 2007). The knowledge and understanding of how ecological factors, both abiotic (like physicochemical conditions, light incidence, temperature, or water retention time) and biotic (organic matter, primary production, viral dynamics, etc.) affect the composition of these microbial communities in freshwater lakes is still very fragmentary (Briée et al., 2007).

Once thought to be restricted to extreme environments, *Archaea* are now found to be virtually in every environment (Brochier-Armanet et al., 2008). Since Woese and collaborators (1990) defined a new domain in the tree of life, *Archaea* have been detected in soils (Nicol et al., 2008), freshwater (Keough et al., 2003; Pascoe and Hicks, 2004), marine coastal (DeLong 1992) and pelagic waters (Fuhrman, 1992), as symbionts in higher organisms (Lange et al., 2005) and even in humans (Vianna et al., 2006). *Archaea* were initially characterized by their ribosomal small subunit (SSU) catalogue, which differed from both the bacterial and the eukaryal catalogues (Woese and Fox, 1977). Upon further study, Woese and collaborators (1978) described the group as lacking peptidoglycan in their diverse, proteinaceous cell walls, having unique tRNA sequences, having L-glycerol ether-linked to branched isoprenoid chains that make a monolayer instead of the commonly known phospholipid bilayer, in addition to the

extremophillic metabolism of the methanogens, sulphur oxidizers, thermoacidophiles and halophiles. Interestingly, archaea share a number of characteristics with Eukarya, like the presence of a TATA box and histones binding the DNA, and have recently been proposed as key players in the endosymbiont theory (Godde, 2012).

In the majority of non-extreme environments, the *Bacteria* have been found to be more abundant and diverse than the *Archaea* (Aller and Kemp, 2008). In the northern Atlantic Ocean, *Archaea* comprise only 20% of the picoplankton, although they increase in relative abundance in waters over 100 m deep (Herndl et al., 2005). Another study found up to 40% of the mesopelagic cells in the Pacific Ocean were archaeal (Karner et al., 2001). In the Laurentian Great Lakes, and other large lake around the world, Keough et al., (2003) found that between 1-10% of planktonic DNA was from archaeal microorganisms. However, new sequences are frequently being identified and further characterized, which suggests that archaeal diversity is far from being understood (Schleper and Nicol, 2010; Barberan et al., 2011; Kubo et al., 2012). In suboxic freshwater ponds, Briée and collaborators (2007) found most of the sequences in their libraries did not correspond to cultured specimens or were only assigned to candidate divisions. Groups previously defined as characteristic of marine or soil environments are increasingly found in other habitats. Most of what is known about archaeal diversity is based on 16S rDNA amplifications and the use of clone libraries (Aller and Kemp, 2008) since researchers have only been able to cultivate a few newly discovered strains in the laboratory (Schleper et al., 2005) and identification to below the phylum level is still very rare (Pires et al., 2012). But new culture-independent techniques allow the sequencing of a whole genome from a single cell (Blainey et al., 2011) and are bringing

the number of fully sequenced archaeal genomes past 100 (Brochier-Armanet et al., 2011). Besides discovering new and diverse lineages of *Archaea*, molecular techniques coupled with traditional cultivation methods have revealed novel pathways of gluconeogenesis and alternatives to the common Calvin cycle (Berg et al., 2010; Walker et al., 2010).

Since 2008, the abundant and ubiquitous mesophilic Crenarchaeota have been reclassified into a new phylum called Thaumarchaeota, basal to the better known Crenarchaeota and Euryarchaeota (Brochier-Armanet et al., 2008). Their sequences were first discovered in the Pacific Ocean, in both coastal (DeLong 1992) and deep water samples (Furham 1992), which earned them the name Marine Group I Crenarchaeota. Further sequencing efforts found that similar organisms likely inhabited soils, sediments and hot springs, with discrete clusters that were named the marine I.1a, soil I.1b and I.1c groups. It became clear not only that mesophilic *Archaea* were more common than initially thought, but that there was a great diversity yet to be characterized (Massana et al., 2000; DeLong, 2003). Shortly after, these mesophilic *Archaea* became the focus of much study, and attempts at cultivation of a representative were finally successful when an autotrophic, ammonia oxidizer was enriched from water in a fish tank and was named *Nitrosopumilus maritimus* (Konneke et al., 2005). Thanks to much genomic study, it is now hypothesized that the Thaumarchaeota might be key players in the nitrogen cycle as ammonia oxidizers (Francis et al., 2007) and the origin of *Eukarya*, or at least share a common ancestor (Gribaldo and Brochier-Armanet, 2006; Williams et al., 2012). Alternatively, it has also been suggested that ammonia-oxidizing *Archaea* (AOA) can

take advantage of fixed forms of carbon, maybe having a heterotrophic pathway (Herndl et al., 2005; Sims et al., 2012).

The Crenarchaeota have now been found also to be ubiquitous and to have a wide array of metabolic capabilities. In a recent study, Kubo and collaborators (2012) identified 17 distinct subgroups of mesophilic *Archaea* within the Miscellaneous Crenarchaeal Group (MCG), that inhabit various environments and that probably play essential roles in sedimentary processes, but little was inferred on the metabolism of these subgroups. The uniquely archaeal process of methanogenesis places the Euryarchaeaota in a key position in the carbon cycle of many environments, being responsible for the anaerobic breakdown of complex organic molecules (Jarrell et al., 2011). This metabolic process has been known since the 1970s although the organisms responsible for it were long misclassified as *Bacteria*. Although the exact details of how this process yields energy are still being investigated (Sanz et al., 2011), the pathway of oxidation of either formate or H_2 , along with the coupled reduction of CO_2 to CH_4 has been well characterized (Kaster et al., 2011). However, new lineages of Euryarchaeota have been recently discovered and not yet associated to nutrient gradients or specific environments that might suggest they utilize this type of metabolism (Barberán et al., 2011; Iverson et al., 2012).

Study system: Oligotrophic lakes

Research into microbial dynamics of freshwater lakes is still lagging far behind that done on oligotrophic ocean systems, even though they are important regulators of nutrients in freshwater before it reaches the ocean (Vissers et al., 2013). Following their

discovery in open oceans, the mesophilic *Archaea* have been found in very heterogeneous freshwater environments, across gradients in temperature, salinity, pH, oxygen and trophic status, amongst other parameters (Auguet et al., 2010; Auguet and Casamayor, 2012; Kubo et al., 2012). Naturally, the heterogeneity in biotic and abiotic factors that influence these ecosystems makes it hard to define general patterns, and limited information is available beyond the detection of nucleic acid sequences.

Oligotrophic lakes are ecosystems of particular interest because their productivity and decomposition rates are at a balance, making them very vulnerable to cultural eutrophication (Cortner and Biddanda, 2002). In these lakes, carbon cycling is based on heterotrophic oxidation of dissolved organic matter (DOM) much more so than in eutrophic lakes, where primary production is more significant (McManus et al., 2003). Heterotrophic organisms play a key role in the microbial food web, and are fundamental in efficient nutrient recycling (Cortner and Biddanda, 2002). Bacterioplankton dominate oligotrophic environments in both numbers and biomass partially because of of their high surface to volume ratio as well as their more efficient growth rates at low nutrient levels (Callieri and Stockner, 2002). It has been documented that picophytoplankton dominate the biomass of great lakes, both tropical and temperate (Guildford et al., 2011 and references therein). Lake Superior is one such system in that it supports 10 times more bacteria per unit of chlorophyll than more eutrophic kettle lakes (Biddanda et al., 2001), which could explain the abundance and distribution of some types of heterotrophic prokaryotes in the water column of Lake Superior (Kish, 2010).

Interestingly, the NO_3^- concentration has increased in the hypolimnion of Lake Superior from 5 μM to 26 μM over the past century (Sterner et al., 2007). Although

nutrient loading into Lake Superior is of concern, studies have shown that the NO_3^- buildup is likely caused by *in situ* nitrification (Finlay et al., 2007) while denitrification is probably hindered by the low organic carbon availability (Small et al., 2013). In a similar fashion, nitrate assimilation is probably impeded by low availability of phosphate, and possibly also by iron limitation (Sterner et al., 2007 and references therein). The increase of nutrient inputs into sensitive ecosystems, and the potential for transport of these nutrients, may affect microbial communities and ultimately alter biogeochemical cycling (Cortner and Biddanda, 2002). Ultimately, nutrient dynamics in Lake Superior could be internally mediated to a great extent by the microbes within it (Small et al., 2013).

The major pathway for nitrogen removal from water is ammonia oxidation coupled with denitrification, where the former is the rate-limiting step (You et al., 2009). Ammonia-oxidizing bacteria (AOB) were known to be critical nitrifiers, but recent studies have found that members of the Thaumarchaeota (previously referred to as mesophilic, or marine group I Crenarchaeota) play a very significant role in nitrification in aquatic (Francis et al., 2005; Konneke et al., 2005) and soil systems (Leininger et al., 2006; Nicol et al., 2008). The ammonia-oxidizing *Archaea* (AOA) described so far have between one and three copies of the *amo*A gene, which codes for the α subunit of the ammonia-monooxygenase enzyme, and can be used to estimate the abundance and activity of ammonia oxidizers (Herrmann et al., 2008). The *amo*A from *Archaea* has been evaluated in a number of marine and estuarine systems, and a positive correlation was detected between abundance of AOA detected by CARD-FISH, and that detected by qPCR assays (Wuchter et al., 2006; Herrmann et al., 2008). In the past few years, AOA

have been detected virtually everywhere and are commonly found to outcompete the ammonia-oxidizing bacteria (AOB). In fact, Wuchter et al. (2006) found the archaeal *amo*A in the North Atlantic was 10 to 1000 times more abundant than the bacteria-like *amo*A. So far, ammonia oxidizers have been ignored as important planktonic primary producers in oligotrophic lakes (see Callieri and Stockner, 2002 for an example) even though their abundance and ubiquity suggest they should be key players in the microbial loop.

Many studies have found the AOA to be especially active at or below the oxygen-minimum zone (OMZ) in the open ocean (Coolen et al., 2007; De Corte et al., 2009) or in the oxic-anoxic boundary (OAB) of freshwater systems (Sims et al., 2012; Vissers et al., 2012). In fact, AOA have been found to grow at faster rates than the AOB in conditions of low ammonia and low oxygen (Martens-Habbena et al., 2009; French et al., 2012). In general, anoxic waters have been greatly understudied, but are thought to be highly diverse in mesophilic *Archaea* and could harbor important communities of AOA (Barberan et al., 2011; Bhattarai et al., 2012).

Lake Malawi: a tropical oligotrophic lake

Sitting south of Lake Tanganyika on the East African Rift Valley, Lake Malawi is the southernmost of the African Great Lakes and an oligotrophic lake. The activity of the African tectonic plate created these rift valley lakes, making them at least 2 million years old, and causing their mountainous shores and deep, long morphometry (Eccles, 1974; Hecky et al., 2003). Some of the morphometric characteristics of Lake Malawi and Lake Superior are quite similar, despite the latter's very different glacial origin (Table 1).

Lake Malawi's surface area accounts for 25% of its catchment, and many of its tributaries run dry during the extended dry season, which leaves rainfall on the lake as the primary water input (Bootsma and Hecky, 2003). Because of its geographical location, Lake Malawi never cools enough to cause lake turnover. Water temperatures typically range between 20 and 30ºC, which leads to significant changes in density with slight changes in temperature, thus creating unstable thermoclines (Bootsma and Hecky, 2003). Water mixing relies on wind energy, precipitation and river inflow, and air temperature. This, along with its great depth, causes Lake Malawi's bottom waters to be permanently anoxic (Eccles, 1974). This slight meromixis leaves the top waters nutrient-depleted, causing the anoxic deep waters to be nutrient rich, and the exchange to be controlled by limited vertical mixing within the lake (Vollmer et al., 2002). Neither the hypolimnetic microbial communities nor the effect that lake mixing might have on them have been studied in Lake Malawi beyond the picoplankton primary producers and N-fixation.

The management of Lake Malawi has historically been an international challenge between the three countries bordering it: Malawi, Tanzania and Mozambique. Unlike the countries bordering Lake Victoria, these three countries do not share a history of colonial government, which makes international collaborations much more recent and communication between researchers and managers somewhat limited (Bootsma and Hecky, 2003). These differences may also explain why research on Lake Malawi's water quality and possibly its microbial communities have been limited, in comparison to research that has been completed on Lake Victoria.

Table 1. Comparison of morphometric characteristics of Lakes Malawi, Kivu and Superior.

Characteristic	Malawi[a]	Kivu[b]	Superior[a]
Surface area (km^2)	29,500	2,370	82,100
Max depth (m)	700	485	407
Mean depth (m)	264	245	149
Volume (km^3)	7,775	560	12,230
Drainage area (km^2)	100,500	5,097	128,000
Altitude (masl)	474	1,460	183
River inflow (km^3/yr)	29	2.4	50
River outflow (km^3/yr)	12	3.6	71
Rainfall (km^3/yr)	39	3.3	65
Evaporation (km^3/yr)	57	3.4	48
Residence time (yr)	114	100	107
Flushing time (yr)	648	160	172

[a] Bootsma and Hecky (2003)
[b] Muvundja et al., (2009)

Research on Lake Malawi has focused on productivity of game fisheries and the highly diverse chiclid fish communities and has only recently shifted towards anthropogenic nutrient inputs and contamination (Bootsma and Hecky, 2003). Although effects of cultural eutrophication have not been as drastic as in Lake Victoria, Lake Malawi has experienced deteriorating water quality, deforestation of its watershed and overfishing (Hecky et al., 2003). The first rains of the year fall on fallow land that is easily eroded, thus increasing nutrient inputs to the lake and causing seasonal patterns for total nitrogen (TN), total phosphorous (TP), and suspended carbon, but not for dissolved organic carbon (DOC; Hecky et al., 2003).

Lake Malawi is considered a seasonally N-limited environment because of its normally low N:P ratios in the water column and the important presence of N-fixing organisms (Gondwe et al., 2008; Guildford and Taylor, 2011). Local and seasonal upwelling is an important source of nutrients to the epilimnion, but is a spatially and temporally limited process (Vollmer et al., 2005). In addition, tropical temperatures and the permanently anoxic hypolimnion lead to high rates of denitrification, thus impeding N accumulation. However, increases in the ratios between N and P have also been reported, and are thought to be caused by recent deforestation and land use changes in the lake's northern catchment, leading to an increase in dissolved inorganic N in the surface water (Hecky et al., 2003). Global climate change is also affecting the lake. Recently, the surface air temperature for the Lake Malawi catchment has been increasing, causing milder winters in the whole region, which is consistent with a deep-water warming trend (Vollmer et al., 2005). The potential effect of these changes on

nutrient cycles could severely impact trophic dynamics in the whole lake and along the length of the food chain (Guildford et al., 2007).

Further north along the rift valley lays Lake Kivu, another meromictic and oligotrophic African great lake with fascinating water characteristics. The lake's bottom waters (below 80 m) contain over 60 km^3 of dissolved methane (CH_4), which is thought to be mostly of biological origin. Due to its low solubility in water, methane contributes more to the gas pressure than the dissolved carbon dioxide (CO_2), with an estimated volume of 300 km^3 (Schimd et al., 2005). Lake Kivu is bordered by the Mitumba Mountains to the west, the Rwandan Dorsal to the east, and to the north by two active volcanoes, Niragongo and Nyamulagira, both of which have erupted in the last 20 years. Any one of these eruptions could trigger a gas release by bringing the gas-saturated bottom water to the surface, and it is believed that a gas release in Lake Kivu would be more lethal than the events at Lakes Monoun and Nyos in the 1980s.

Microbiology of the Laurentian Great Lakes

Although much remains to be investigated, the microbes in Lake Superior have been the focus of a number of studies (Reed & Hicks, 2011). Pascoe and Hicks (2004) found 2% of the total nucleic acids isolated from sites in the Laurentian Great Lakes were archaeal. More specifically for Lake Superior, Keough et al. (2003) reported that up to 5% of picoplanktonic DNA came from the *Archaea*. These numbers, as well as estimates for other great lakes around the world, correspond to a smaller proportion of planktonic archaeal to total picoplankton DNA than that found in marine environments (Keough et al., 2003). However, little is known about the factors that determine archaeal

distribution in the water column. Kish (2010) focused on one site in Lake Superior, where he found archaeal abundance varied temporally in the water column. This pattern has been seen in other temperate lakes that undergo thermal stratification (Auguet and Casamayor, 2008; Vissers et al., 2013). Similarly, differences in community composition and abundance of cells between surface and deep waters suggested that mesophilic *Archaea* thrive in the deep cooler waters, leading to a loss of species and lower abundances in the warmer surface waters (Kish, 2010). MacGregor et al. (1997) found aerobic methanogens in the deep hypolimnion of Lake Michigan, as well as a peak in abundance of what they called mesophilic Crenarchaeota (now identified as Thaumarchaeota) just below the oxicline. On a spatial scale, it has been reported that picoplankton community DNA was more than 70% similar in surface waters (5 m) along the Laurentian Great Lakes (Pascoe and Hicks, 2004) but other spatial patterns in prokaryotic abundance and diversity have not been investigated with a higher resolution in the Laurentian Great Lakes.

The Laurentian Great Lakes have experienced human development on their shores for several centuries, and much more is known about the effects of nutrient loading and changes in their watersheds compared to the African great lakes, where bordering countries are rapidly developing. Lake Malawi has frequently been compared to Lake Superior because of the similarities in their granitic geology, clear waters, great size and their extremely oligotrophic condition (Guildford et al., 2000; Hecky, 2000). The planktonic microbial communities of these lakes might be similar because of their trophic status. Llirós and his collaborators (2010) found a similar distribution pattern for archaeal abundance in Lake Kivu compared to that seen in Lake Superior (Kish 2010),

with lower numbers in the surface waters and a distinct peak below the thermocline. The goals of this study were to determine if the distribution of archaeal abundance in the water column of a tropical, oligotrophic lake was similar to patterns seen in other studies of temperate and tropical oligotrophic great lakes, to describe spatial and temporal variations in the abundance of total *Archaea*, MG-1 *Archaea*, and AOA, and to compare the diversity of planktonic *Archaea* in Lakes Malawi, Kivu, and Superior.

Archaeal cellular memberanes are unlike those of Eukarya or Bacteria in that there is a monolayer with C_{40} isopropenoid side chains. Particular to the Thaumarchaeota are the glycerol dialkyl glycerol tetraethers (GDGTs) with multiple cyclopentane moieties that are proportional to the growth temperature (Ingalls and Pearson 2013). Because of their high preservation potential of around 100 million years, and their ubiquity in oxic and anoxic environments, these lipids have been used as paleotemperature proxies in aquatic sediments (Ingalls & Pearson, 2013). Leininger and collaborators (2006) found crenarchaeol correlated with the abundance of *amo*A in soils. Woltering and collaborators (2012) evaluated the TEX_{86} proxy, based on Thaumarchaeotal GDGTs as indicators of subsurface water temperature in Lake Superior, and found sediment samples agreed with the observed *in situ* temperatures.

CHAPTER II
Planktonic Archaeal Abundance and Diversity Change with Depth in Lakes Malawi and Kivu

Introduction

The *Archaea* are now recognized as an important component of the bacterioplankton in lakes (Keough et al., 2003; Lliros et al., 2010) and even rival the *Bacteria* in abundance in the deep ocean (Karner et al., 2001). However, the ecological significance of all types of *Archaea* in mesophilic aquatic systems has yet to be fully understood (Barberan et al., 2011; Vissers et al., 2013). Initially referred to as the marine mesophilic Crenarchaeota (DeLong 1992; Furham 1992; Francis et al., 2005), the marine group I.1a, and the soil-associated groups 1b and 1c are now classified into the Thaumarchaeota. Very few cultured specimens exist within this new phylum, and so they have mostly been characterized by sequences from fosmid libraries and the environmental conditions in which these sequences are repeatedly found (Schleper et al., 2005). It is now accepted that most of the organisms within the Thaumarchaeota have the potential to oxidize ammonium through the detection of a putative gene for the α subunit of the ammonia monooxygenase (commonly referred to as the *amo*A). Some recent studies found that Thaumarchaeota thrive and outcompete ammonia-oxidizing bacteria (AOB) in low nutrient aquatic environments (Martens-Habbena et al., 2009; Reed et al., 2010), making them an important group to target in Lake Malawi. Ammonia-oxidizing archaea (AOA) are ubiquitous in both aquatic and soil environments, which suggests they play an essential role in N cycling (Francis et al., 2007; Reed et al., 2010). Of particular interest are the members of the Marine Group I (MG-I) *Archaea* that have been found in lakes

around the world and are likely the main nitrifiers in oligotrophic environments (Kish, 2010; Auguet et al., 2012).

Lake Malawi has lower total nitrogen (TN) and phosphorous (TP) in its euphotic zone than the Sargasso Sea or Lake Superior, which is famously the lowest-nutrient temperate great lake (Guildford et al., 2007). Because of its great depth and tropical temperatures, Lake Malawi is permanently stratified with anoxic waters below ~200 m that never come into contact with the atmosphere. This deep hypolimnion is a source of nutrients that only occasionally surface due to upwelling, turbulence between the water masses, or slow diffusion (Bootsma and Hecky, 2003). The fast growing populations and economies of Malawi, Tanzania and Mozambique depend on Lake Malawi for food, tourism and transport, with the fisheries being an important economic driver in the region. Land use shifts and global climate change are clear threats to the stability of this ecosystem, and much research is still needed to guide sound management (Bootsma and Hecky, 1993; Hecky et al., 2003).

Denitrification is believed to be the most important pathway for nitrogen removal from Lake Malawi, but this process has not been accounted for in nutrient budgets (Bootsma and Hecky 1999). Ammonia oxidation is the rate-limiting step for this nitrogen removal. AOA have higher growth rates and outnumber the AOB in oligotrophic enrichment experiments, and to date, their *amo*A gene has the highest affinity for NH_4^+ of the cultured ammonia oxidizers (Martens-Habbena et al., 2009), suggesting they may be the key organisms in ammonia removal in oligotrophic systems.

Methanogenic *Archaea* are common in freshwater environments and may play an important role in carbon cycling by aiding in the decomposition of complex organic

molecules (Bridgham et al., 2013). They have been found in the sediments (Bhattarai et al., 2012) and in the anoxic water column (Lliros et al., 2008; Lliros et al., 2010) of tropical and temperate lakes. However, environmental sequences that form many new clades within the Euryarchaeota have been discovered recently, without much indication of the metabolic capabilities of their members or their association to particular nutrient cycles (Glissman et al., 2004). These sequences are also consistently diverse, especially in freshwater environments, suggesting a great potential for novel metabolic characteristics (Barberan et al., 2011).

The objectives of this work were to establish the vertical distribution and evaluate the diversity of planktonic *Archaea* in the water columns of Lakes Malawi and Kivu during thermally stratified conditions. Unfortunately, the microbiology of Lake Malawi has not been investigated beyond the photoautotrophs and diazotrophs in the epilimnion. Thus, results from this study are the first examination of planktonic archaeal populations and diversity in Lake Malawi. Lake Kivu has been the focus of a detailed study into the abundance and diversity planktonic *Archaea* (Llirós et al., 2010) and another study that describes sediment *Archaea* (Bhattarai et al., 2012). These two investigations are the only ones that have focused on the *Archaea* in tropical lakes before this study.

Methods

Lake Malawi sites and sampling

Situated at the south of the inter-tropical convergence zone, Lake Malawi experiences a monsoon-like climate with one well defined annual warm and rainy summer from December to April (Pilskaln, 2004), followed by a cool, dry winter that causes some mixing from May to August, and a wet, cool and mixed period from

September to November (Vollmer et al., 2002). There are marked gradients in rainfall, river discharge and productivity from north to south, which is why we attempted to sample the length of the lake. The southeastern arm, around 80 km long, is the shallowest and coolest part of the lake because of strong southeasterly seasonal winds called Mwera, which cause a fair amount of upwelling (Vollmer et al., 2002). Therefore, the South Basin is more productive than the other two basins of Lake Malawi (Fig. 1) and it is frequently oxygenated throughout the water column. The land surrounding the South Basin is the most densely populated area of the Lake Malawi, where land use has shifted toward agriculture, and is less wooded than the northernmost part of the lake (Pilskaln, 2004). The Central Basin is the deepest part of the lake, at just over 700 m deep. Because of their greater depth, the Central and North basins have permanently anoxic waters below 200 m. The North Basin, being closer to the equator, periodically experiences longer rainy seasons than the rest of the lake (Nicholson, 2000) and the steeper slopes of the mountains surrounding the lake pointing this region contribute to greater runoff.

Each of Lake Malawi's basins (North, Central and South) was sampled twice (Fig. 1, Table 2). All basins (NB, CB, and SB sites) were sampled from January 9-13, 2010 aboard the *R/V Ndunduma*. Different sites were sampled using the *R/V Ndunduma* or smaller boats in the North (Tchambo), Central (Nkhata Bay), and South (Senga Bay) Basins on January 2011, January 2012, and November 2011, respectively (Table 2). All sites but one (Senga Bay, South Basin) were sampled during the rainy summer (January 2010-2012), which causes a deep thermocline, at around 30 m. The Senga Bay site was

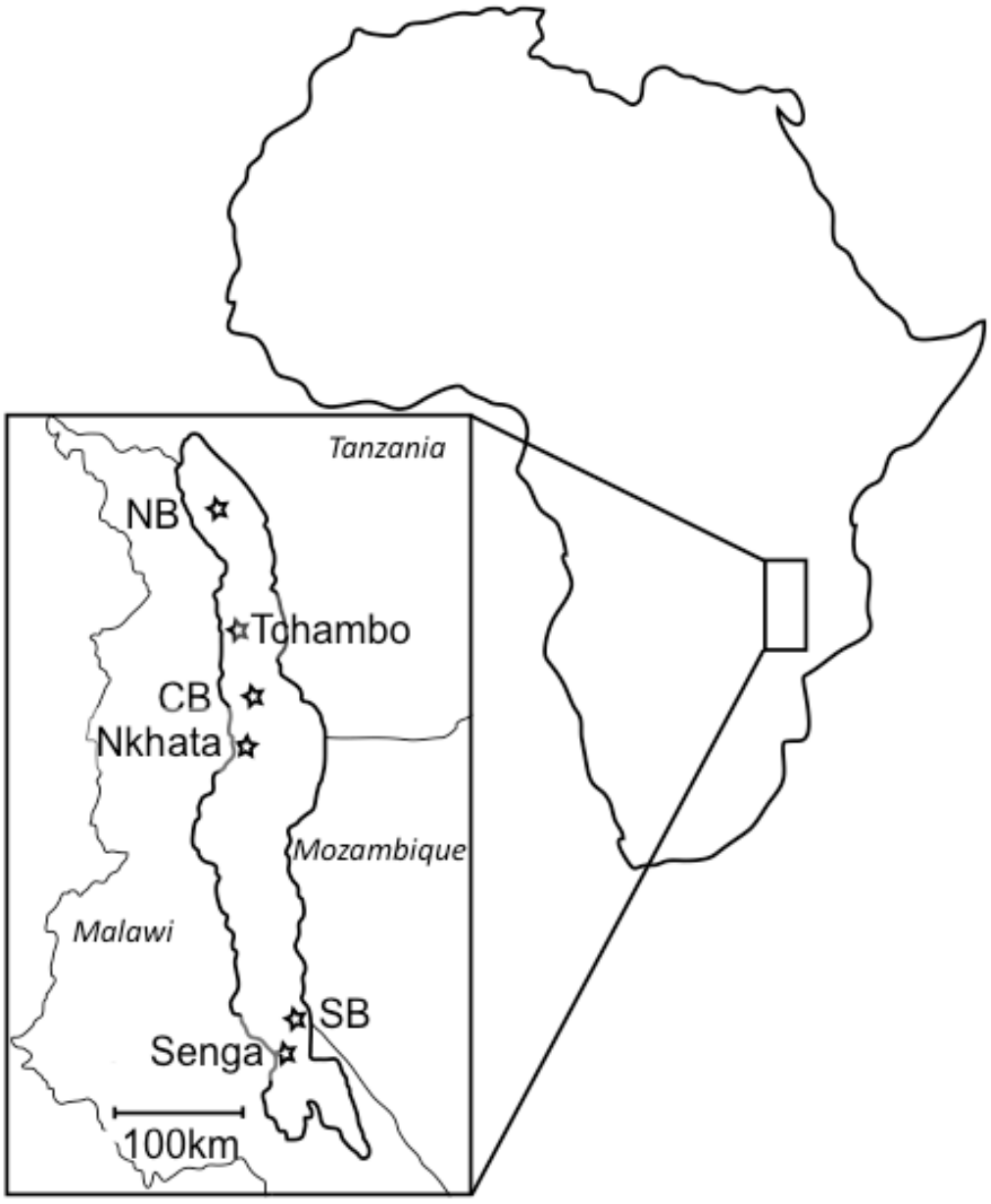
Tanzania
NB
Tchambo
CB
Nkhata
Mozambique
Malawi
SB
Senga
100km

Fig 1. Locations of sites sampled in Lake Malawi between January 2010 and January 2012.

Table 2. Site names and locations sampled in Lake Malawi and Lake Kivu during 2010, 2011, and 2012.

Lake	Year	Month	Day	Site	Latitude / Longitude	Depths (m)
Malawi	2010	Jan	9 to 13	NB (Abbot 2001 core MAL05-2A)	10°01.1'S, 34°11.2'E	10, 30, 50, 100, 150,
				CB (Abbot 2001 core MAL05-1C)	11°17.7'S, 34°26.2'E	200, 300
				SB (station 900, Bootsma and Hecky, 1999)	13°28.7'S, 34°44.8'E	
	2011	Jan	13	Tchambo (North Basin)	10º50.4'S, 34º20.3'E	10, 40, 140, 230, 500
	2011	Nov	13	Senga Bay (Station 928, South Basin)	13º42.8'S, 34º40.4'E	10, 30, 50, 100, 150
	2012	Jan	12	Nkhata Bay (Central Basin)	11º35.2'S, 31º21.0'E	10, 25, 50, 300
Kivu	2012	Jan	11	K10 (Guitende Bay)	02º06.6'S, 29º16.2'E	3, 20
			13	K11 (Offshore)	02º03.2'S 20º15.1'E	60, 100

sampled in late November where the water column was also thermally stratified at the time of sampling.

Lake water was collected using 8L Niskin bottles at several depths in the epilimnion, hypolimnion and below the chemocline at each site. Water column profiles of temperature and chlorophyll *a* concentrations were obtained using a SeaBird Model 19® plus CTD with fluorometer, DO, pH, and water pressure sensors. During the 2010 cruise, water was filtered onboard the *R/V Ndunduma*. Water samples collected at other times were brought to shore for filtering in a laboratory. As soon as possible after collection, all water samples were vacuum-filtered onto Millipore Durapore filters (47 mm dia.; 0.22 μm-pore) until the filter clogged, placed into Whirl-Pak bags, and stored frozen (-20°C) before being shipped to the University of Minnesota Duluth. Upon arrival, all filters were stored at -80ºC until DNA could be extracted.

Lake Kivu sites and sampling

Lake Kivu is a small oligotrophic great lake in the mountains between Rwanda and the Democratic Republic of the Congo (DRC) that has bottom water that is supersaturated with CO_2 and CH_4^-. It is the only African lake where archaeal abundance, diversity and distribution has been previously investigated (Lliros et al., 2010; Bhattarai et al., 2012). Water samples were obtained on the 11 and 13 of January 2012 using a Niskin bottle at site K10 off the shore of Guitende Bay, or further in-lake at site K11 and returned to a field laboratory (Table 2). In the lab, water samples from each depth were vacuum-filtered onto Durapore membrane filters (47 mm dia., 0.22 μm-pore; Millipore Corp.) as described above. Filters containing microbial cells were placed in

Whirlpakbags, frozen where possible, and then shipped to the lab at the University of Minnesota Duluth between ice packs soon after.

DNA Extraction

Total DNA was isolated using a PowerWater DNA Isolation kit (MoBio Laboratories, Inc.) according to the manufacturer's instructions and eluting the DNA in the supplied elution buffer. Nucleic acid concentrations were determined using a NanoDrop 3000 fluorospectrometer (Thermo Scientific Inc.) with Quant-iT Picogreen dye (Invitrogen). Isolation yields were estimated to be 21% after spiking filters with known concentrations of *E. coli* DNA and performing 3 separate isolations.

Quantitative PCR Analyses to Estimate Total Archaeal, MG-I, and Archaeal amo*A Abundances*

Quantitative polymerase chain reaction (qPCR) assays using Brilliant II SYBR Master Mix (Agilent Technologies) were performed with DNA extracted from lake water picoplankton to estimate the abundance of different archaeal groups [*i.e.*, total , Marine Group I *Archaea*, and ammonia-oxidizing *Archaea* (AOA)] (Table 3). All qPCR reactions were conducted using a Corbett Research Rotor-Gene 3000 thermal cycler. Standards for the qPCR reactions were serial dilutions ranging from 20 pg/μL to 2 ag/μL of gene fragments amplified from PCR products of appropriate Lake Superior clones (Su10h3a referenced in Keough et al., 2004 for total and MG-I *Archaea*, and clone 40m3C3 isolated in R.E. Hicks' lab for the archaeal *amo*A assay). These fragments were initially amplified using the M13 primers supplied with the cloning kits (see below). qPCR reactions were performed in triplicate for each DNA sample, and gene copies were quantified using linear regression and a standard curve relating C_t values to gene

Table 3. qPCR primers and cycling parameters used to estimate the abundance of total *Archaea*, Marine Group I *Archaea*, and ammonia-oxidizing *Archaea* (amoA gene) for all assays performed.

Target	Primer	Sequence (5' – 3')	Program	Reference
Archaeal 16S rDNA (396bp)	PARCH519f	CAG CCG CCG CGG TAA	30 sec, 95ºC 30 sec, 63ºC	Coolen et al., 2004
	ARC915r	GTG CTC CCC CGC CAA TTC CT	30 sec, 72ºC 15 sec, 81ºC *	
Archaeal *amo*A (635bp)	Arch-amoAF	STA ATG GTC TGG CTT AGA CG	30 sec, 95ºC 30 sec, 53ºC 1 min, 72ºC	Francis et al., 2005
	Arch-amoAR	GCG GCC ATC CAT CTG TAT GT	20 sec, 78ºC *	
Marine Group I (163bp)	MCG1-391F	AAG GTT ART CCG AGT GRT TTC	30 sec, 94ºC 40 sec, 61ºC 40 sec, 72ºC	Takai and Horikoshi, 1999 Teira et al., 2004
	MCG1-554R	TGA CCA CTT GAG GTG CTG	20 sec, 78ºC *	Lliros et al., 2010

* Data acquired

All programs were 40 cycles preceded by a 10 minute hold at 95ºC (except MG-I, which was 45 cycles with a 4 min hold at 94ºC) followed by a gradual increase of temperature from 72ºC to 95ºC by 1º steps, with a 45 sec wait on the first step, and 5 sec wait on consecutive steps.

copies (typically 10^1 to 10^8 gene copies). No-template control and negative control reactions containing a non-target sequence (*Pseudomonas fluorescens* total DNA) were used to check the specificity of the PCR reactions.

Cloning and sequencing

Picoplankton DNA samples from the CB site in Lake Malawi were selected for cloning and sequencing analysis. Samples from 10, 100 and 300 m were selected from the oxic epilimnion, oxic hypolimnion, and anoxic hypolimnion of this lake, respectively. Picoplankton DNA from 20 m (site K10) and 60 m (site K11) in Lake Kivu were chosen because the 20 m depth is in the oxic epilimnion and the 60 m depth is within the upper part of the anoxic hypolimnion of this lake. Archaeal DNA was amplified in a portion of the 16S rRNA gene using primers PARCH519r and ARC915r (Table 3). PCR products from each DNA sample were then cloned into One Shot® competent E. coli cells using a TOPO TA cloning kit following the manufacturer's instructions (Life Technologies). Plasmids were extracted and isolated with a UltraClean® Mini Plasmid Prep kit (MoBio Laboratories, Inc.) and the partial 16S rDNA inserts were sequenced at the Biomedical Genomics Center (University of Minnesota) using the PARCH519f primer.

Sequences from each sample were then analyzed for quality and trimmed to the correct size (396 base pair maximum) using 4Peaks version 1.7.2 (Griekspoor and Groothuis, 2005) and a chimera check was performed using MALLARD (Ashelford et al., 2006). All sequences that were similar to reported archaeal sequences found in GenBank using BLAST (http://blast.ncbi.nlm.nih.gov) were retained for further analysis

and deposited in Genbank (accession numbers KF274693 – KF274957). The sequence database for these clones was further enriched with sequences of cultured strains and environmental sequences that were representative of freshwater environments. Sequence alignments and phylogenetic trees were obtained using MEGA version 5.0 for Mac (Tamura et al., 2011) with the CLUSTAL W and neighbor-joining algorithms with 1000 bootstrap replicates. Fastgroup II (Yu et al., 2006) was used to group sequences into operational taxonomic units (OTUs) at 97% sequence identity and to perform rarefaction analysis, which allowed for richness comparison among unequally sampled environments.

Statistics

The effects of depth on gene abundances were evaluated with a one-way ANOVA with an alpha value of 0.05. Spearman's correlation was performed on the *amo*A gene abundance versus the MG-I and total *Archaea* 16S rDNA abundances, as well as between gene copy numbers and temperature. All analyses were performed using JMP version 7 (SAS Institute Inc.).

Results

The water column in Lake Malawi was thermally stratified in all basins and during each sampling period, with temperatures that ranged from 28ºC at the surface to 23ºC below the thermocline (Fig. 2), which extends from the surface to 20-80 m depending on the site. All sites but the SB site had hypoxic water, under 40% saturation, below 100 m. A deep chlorophyll layer (DCL) was present between 20 and 50 m at all sites for which CTD and fluoroprobe data was available, with a sharp peak just under 50 m. Prokaryotic

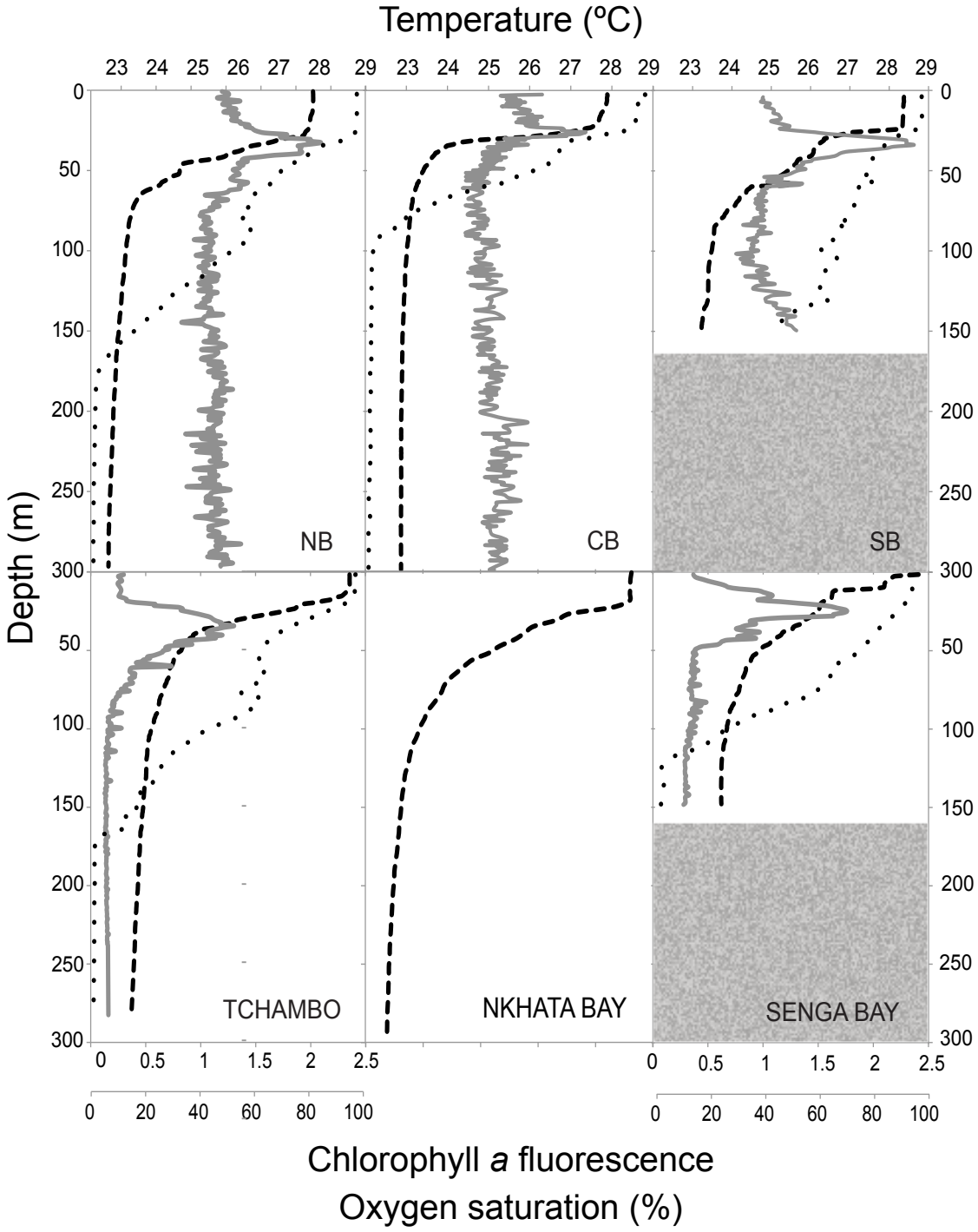
Temperature (ºC)
23 24 25 26 27 28 29
Depth (m)
0 50 100 150 200 250 300
NB
CB
SB
TCHAMBO
NKHATA BAY
SENGA BAY
0 0.5 1 1.5 2 2.5
0 20 40 60 80 100
Chlorophyll a fluorescence
Oxygen saturation (%)

Fig. 2. Temperature (dashed line), dissolved oxygen (dotted line), and chlorophyll *a* (solid line) profiles in Lake Malawi when water samples when collected for microbiological analyses. The top panels show profiles of these parameters at sites in the North (NB), Central (CB) and South (SB) basins sampled in January 2010. The bottom panel shows profiles for the North (Tchambo) and South (Senga Bay) basins that were sampled in January and November 2011, respectively. Only temperature profiles were available for the Nkhata Bay site. The shaded area illustrates the maximum depth at the site.

cell abundances using DAPI staining ranged from 2.94 (± 0.65) × 10^8 to 2.31 (± 1.42) × 10^9 cells/L, with numbers in surface waters (10 and 30 m) being greater than those from the deeper water. These values are consistent with abundances reported in other African (Lliros et al., 2010) and Laurentian Great Lakes (Hicks et al., 2004).

One goal of this study was to evaluate the differences in the spatial and temporal abundance of several archaeal groups, so sample sites in each basin of Lake Malawi were visited during the thermally stratified period over two years (Table 2). Two Lake Kivu sites (Table 2) were also sampled one time during the thermally stratified period. Generally, the qPCR profiles for total *Archaea* and the archaeal *amo*A gene in the three basins of Lake Malawi followed a similar pattern of low abundance in epilimnion and a rapid increase of several orders of magnitude in abundance in the upper hypolimnion (Fig. 3). The abundance of total *Archaea* increased sharply beneath the thermocline (50 m) and remained at least one order of magnitude higher throughout the hypolimnion ($p < 0.01$). Similarly, the archaeal *amo*A gene abundance was significantly lower in the epilimnion and at least an order of magnitude more abundant below 50 m (t test, $p=0.009$). At most sites, the MG-I archaeal abundance was usually below the detection limit of the qPCR assay (~200 copies/L) in the epilimnion (above 50 m) and their abundance peaked closer to the deeper oxygen chemocline than to the thermocline.

The patterns of total *Archaea*, MG-I, and archaeal *amo*A gene abundance were similar at the two sites sampled in the North Basin (NB and Tchambo) of Lake Malawi during 2010 to 2011 (Fig. 3). Even though the archaeal *amo*A gene abundance seemed

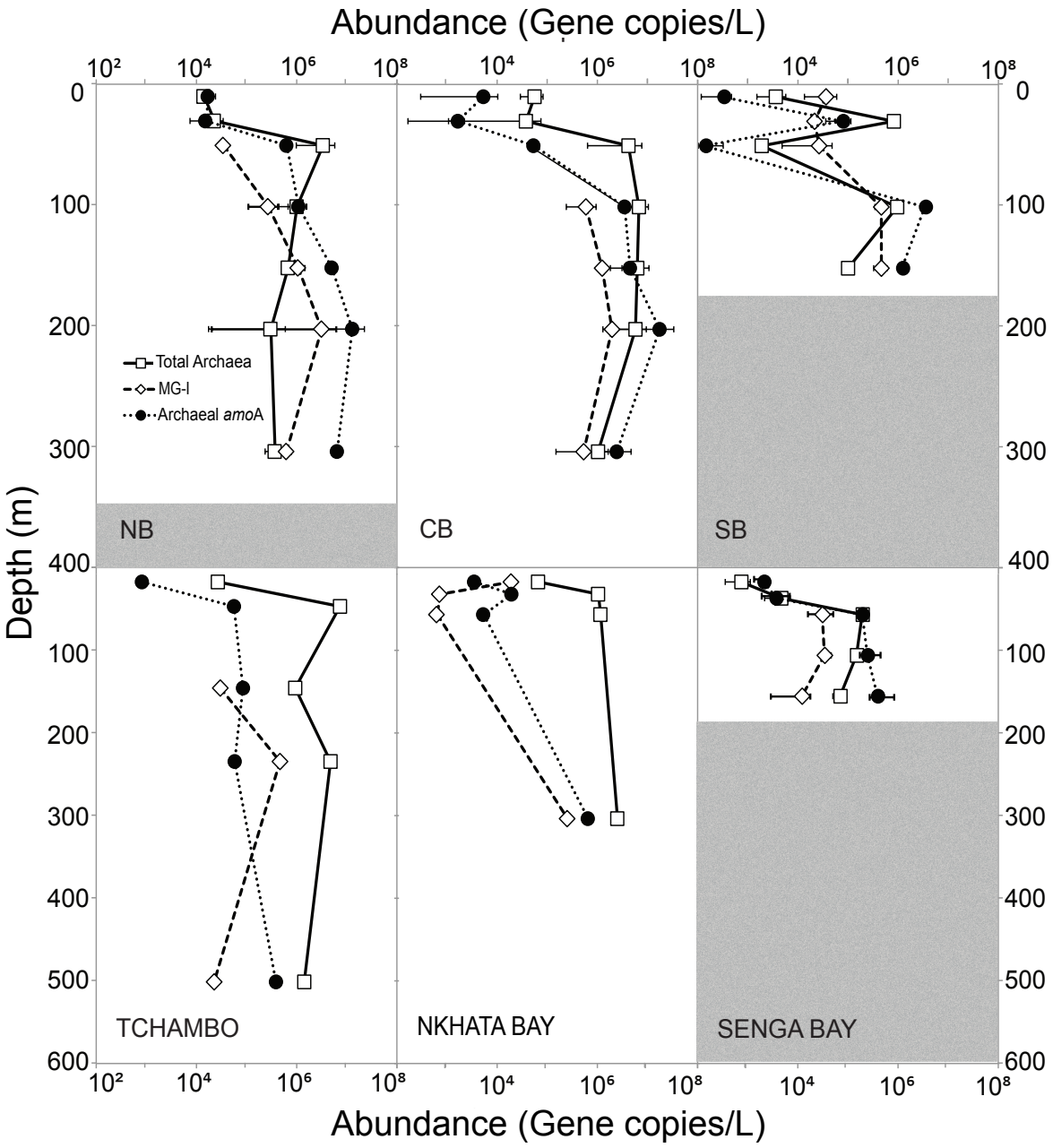
Abundance (Gene copies/L)
Depth (m)
Total Archaea
MG-I
Archaeal amoA
NB
CB
SB
TCHAMBO
NKHATA BAY
SENGA BAY
Abundance (Gene copies/L)

Fig. 3. Vertical distribution of archaeal 16S rDNA (open squares), Marine Group I (open diamonds, dashed line), and the archaeal ammonia monooxygenase A gene (*amo*A; closed circles, dotted line) copy numbers in the water column of the North, Central and South basins (left to right) of Lake Malawi during 2010 (top panel) and 2011/2012 (bottom panel). The shaded area represents maximum depth at the sampling sites. Error bars represent means ± SE (of replicate samples; Tchambo and Nkhata Bay samples were not replicated).

less at Tchambo compared to NB site, there was no significant difference ($p = 0.085$). At the NB site, the abundance of the archaeal *amo*A and the MG-I archaea increased below 100m and peaked near the oxic-anoxic boundary, while the total archaeal abundance decreased below 50 m and was significantly less abundant below the oxic-anoxic boundary ($p < 0.05$). The archaeal *amo*A and MG-I profiles did not match as closely at the Tchambo site when compared to the NB site. Although total archaeal abundance increased from the epilimnion to hypolimnion at the two Central Basin sites (CB and Nkhata Bay) in Lake Malawi, there were differences between the amoA and Mg-I profiles at these sites (Fig. 3). Although the MG-I were undetectable above 100 m at the CB site, the profiles of the three archaeal markers displayed similar patterns at this site. At Nkhata Bay, however, total archaeal and archaeal *amo*A abundances increased from the surface to 25 m and increased further in the deep hypolimnion (300 m), while the MG-I abundance decreased from the 10 to 50 m before rising at 300 m in the deep hypolimnion. The abundance of MG-I was higher in the epilimnion than in the metalimnion at Nkhata Bay (t test, $p<0.01$), a pattern that was less pronounced at the SB site, the only other site where MG-I abundance was detectable in the epilimnion.

Although archaeal gene abundances were generally higher in the hypolimnion than epilimnion at the two sites in the South Basin (SB and Senga Bay), the profile patterns were not alike, just as in the Central Basin. The profiles of total archaeal, MG-I, and archaeal *amo*A abundance at the Senga Bay site were similar to profiles observed at the NB and CB sites in 2010 and Tchambo in 2011. At the SB site in the South Basin, however, the total archaeal and archaeal *amo*A profiles displayed erratic changes that

were atypical of the MG-I gene marker and profiles at other sites in Lake Malawi. The abundance of these groups increased approximately 100-fold from 10 m to 25 m, decreased by similar amounts at 50 m, and then increased by almost 3 orders of magnitude at 100 m. The MG-I and archaeal *amo*A abundances were highest below 100 m at the SB site, but still in the oxic portion of the hypolimnion. Interestingly, the archaeal *amo*A profile at SB did not follow the MG-I profile as it did at the NB and CB sites sampled in 2010 but instead followed the erratic pattern observed for total archaeal abundance (Fig. 3).

The abundance of all archaeal groups in Lake Kivu was within the range of numbers found in Lake Malawi (Table 4). Prokaryotic cell counts showed higher abundance of cells in the surface, and decreased below 20 m, as was seen in Lake Malawi (Appendix). The total *Archaea* increased with depth to a maximum at 60 m, while the MG-I abundance oscillated and the *amo*A gene abundance remained fairly stable. However, due to lack of proper replication, no statistical tests were done.

Combined, 260 partial 16S rRNA gene sequences (156 from Lake Malawi, 104 from Lake Kivu) were analyzed and 164 OTUs were identified (Fig. 4). Most sequences were recovered from hypolimnetic waters of Lakes Malawi and Kivu. None of the archaeal sequences recovered were anomalous or chimeric. The majority of archaeal sequences recovered from the oxic hypolimnion of Lake Malawi were related to the Thaumarchaeota, while those from the anoxic hypolimnia of both Lakes Malawi and Kivu were of euryarchaeal origin (Fig. 4). The deep samples in each lake had greatest

Table 4. Abundance of total *Archaea*, MG-I *Archaea*, and the archaeal *amo*A gene at two sites in Lake Kivu sampled in January 2012.

Site	Depth (m)	Abundance (gene copies/L)			DAPI (cells/mL)
		Total Archaea	MG-I	*amo*A	
K10	3	1.53E+05	8.92E+03	2.75E+03	1.21E+09
	20	2.33E+05	1.72E+05	3.20E+03	1.99E+09
K11	60	1.52E+06	3.25E+04	1.25E+03	8.91E+08
	100	6.28E+07	4.20E+05	7.28E+03	6.02E+08

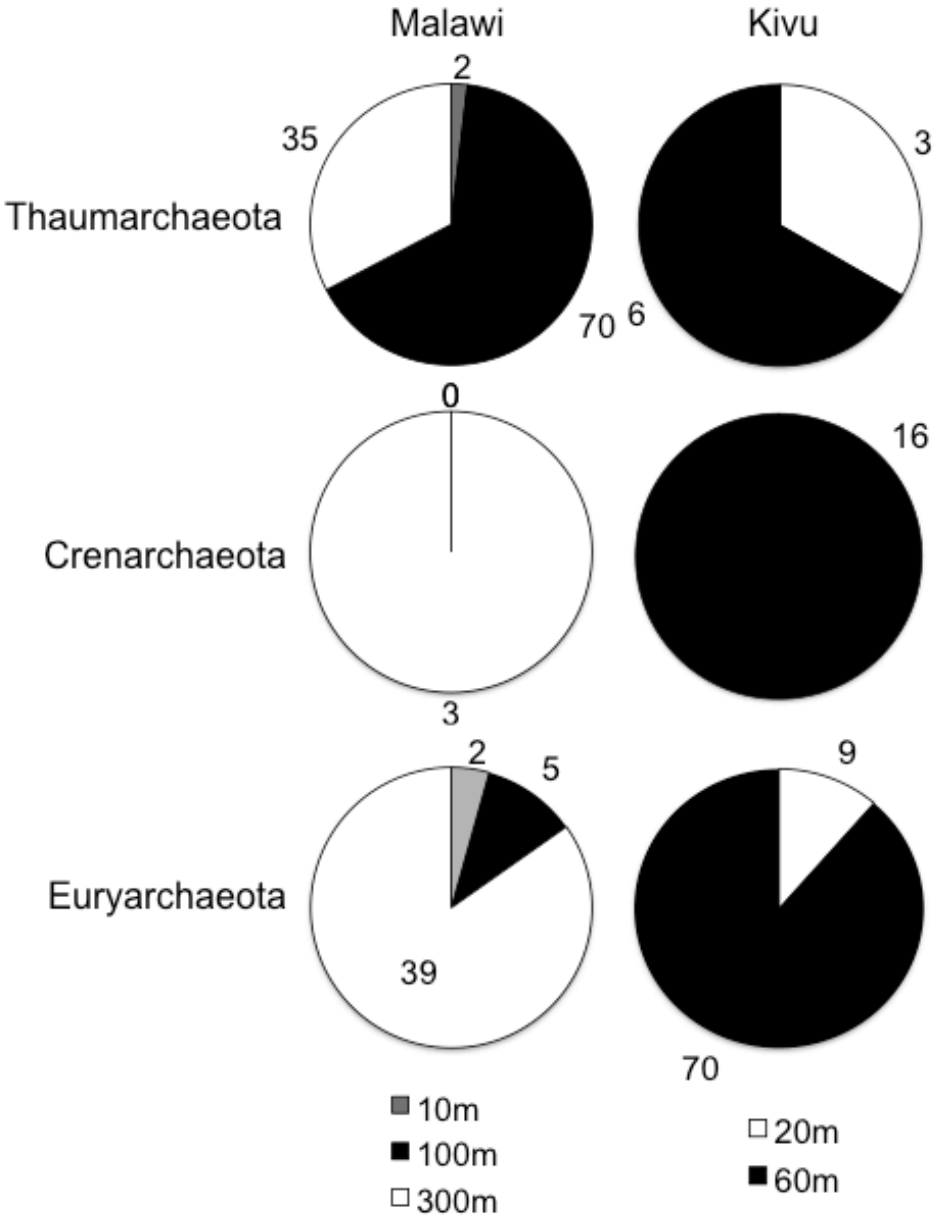
Malawi
Kivu
Thaumarchaeota
Crenarchaeota
Euryarchaeota
2
35
70
3
6
0
3
16
2
5
39
9
70
10m
100m
300m
20m
60m

Fig. 4. Number of partial 16S rRNA gene sequences recovered from Lakes Malawi and Kivu that were related to the Thaumarchaeota, Crenarchaeota, and Euryarchaeota.

number of unique sequences and the highest diversity according to rarefaction analysis (Fig. 5). Very few archaeal sequences were recovered from the oxic surface waters of Lakes Malawi (4 of 80 clones at 10 m) and Kivu (12 of 50 clones from 20 m). Interestingly, the majority of epilimnetic clonal sequences appeared to be bacterial, primarily from the genera *Sphingobacterium* and *Synechoccocus*. Some bacterial sequences were found at deeper depths in each lake but not in such high proportions (26% for 300 m in Lake Malawi; 16% for 100 m and 60 m in Lakes Malawi and Kivu, respectively) as in the epilimnia of these lakes.

Most of the archaeal sequences clustered amongst the Euryarchaeota (48%), closely followed by the Thaumarchaeota (45%), and fewer sequences were related to the Crenarchaeota (7%; Fig. 4). Most of the euryarchaeotal sequences were found in the anoxic hypoliminia of Lake Kivu (70 sequences at 60 m) and Lake Malawi (39 sequences at 300 m). These depths are typically within the anoxic portions of the hypolimina of these lakes (Fig. 2, Lliros et al., 2010, Schmid et al., 2005). The vast majority of Thaumarachaeota sequences were found in oxic (100 m, 70 sequences) and anoxic (300 m, 35 sequences) hypolimnetic waters of Lake Malawi. All archaeal sequences related to the Crenarchaaeota were from deeper anoxic parts of the hypolimnia in Lakes Malawi (300 m) and Kivu (60 m). Most of the thaumarchaeotal sequences were affiliated with the Marine Group I (Fig. 6A) and similar to archaeal sequences previous identified in Lake Victoria (37 sequences; Keough et al., 2003) and the Laurentian Great Lakes (18 sequences; Keough et al., 2003, Kish 2010). Three Thaumarchaeotal sequences fell within the archaeal Soil Group I.1b.

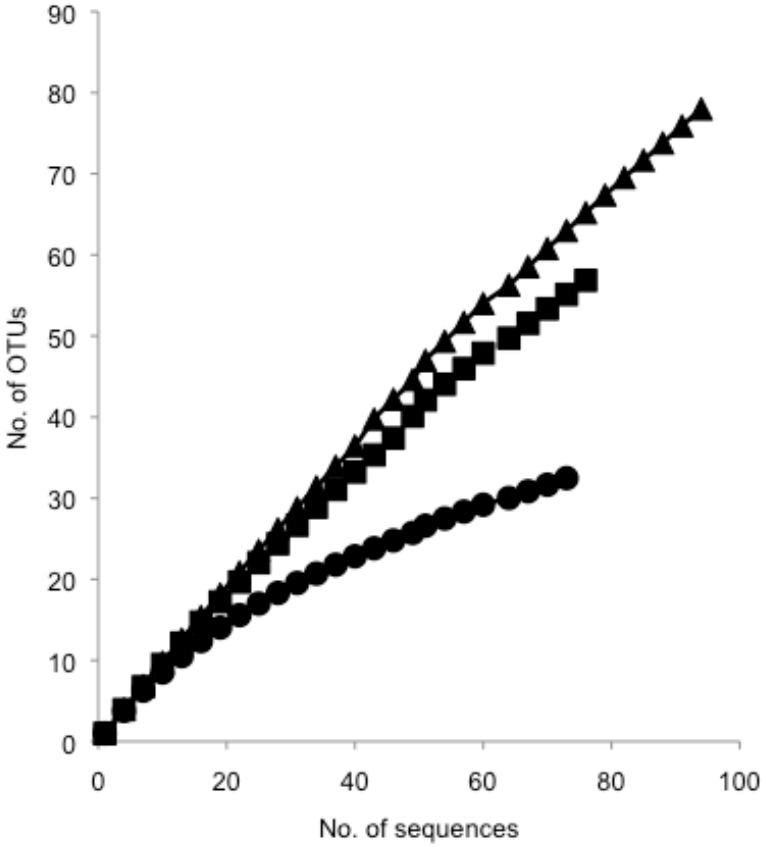
90
80
70
60
50
40
30
20
10
0
No. of OTUs
0
20
40
60
80
100
No. of sequences

Fig. 5. Rarefaction curves of unique operational taxonomic units (OTUs; 97% sequence identity) for 16S rDNA gene clone libraries from the water column of Lake Malawi (100 m, circles; 300 m, squares) and Lake Kivu (60 m; triangles).

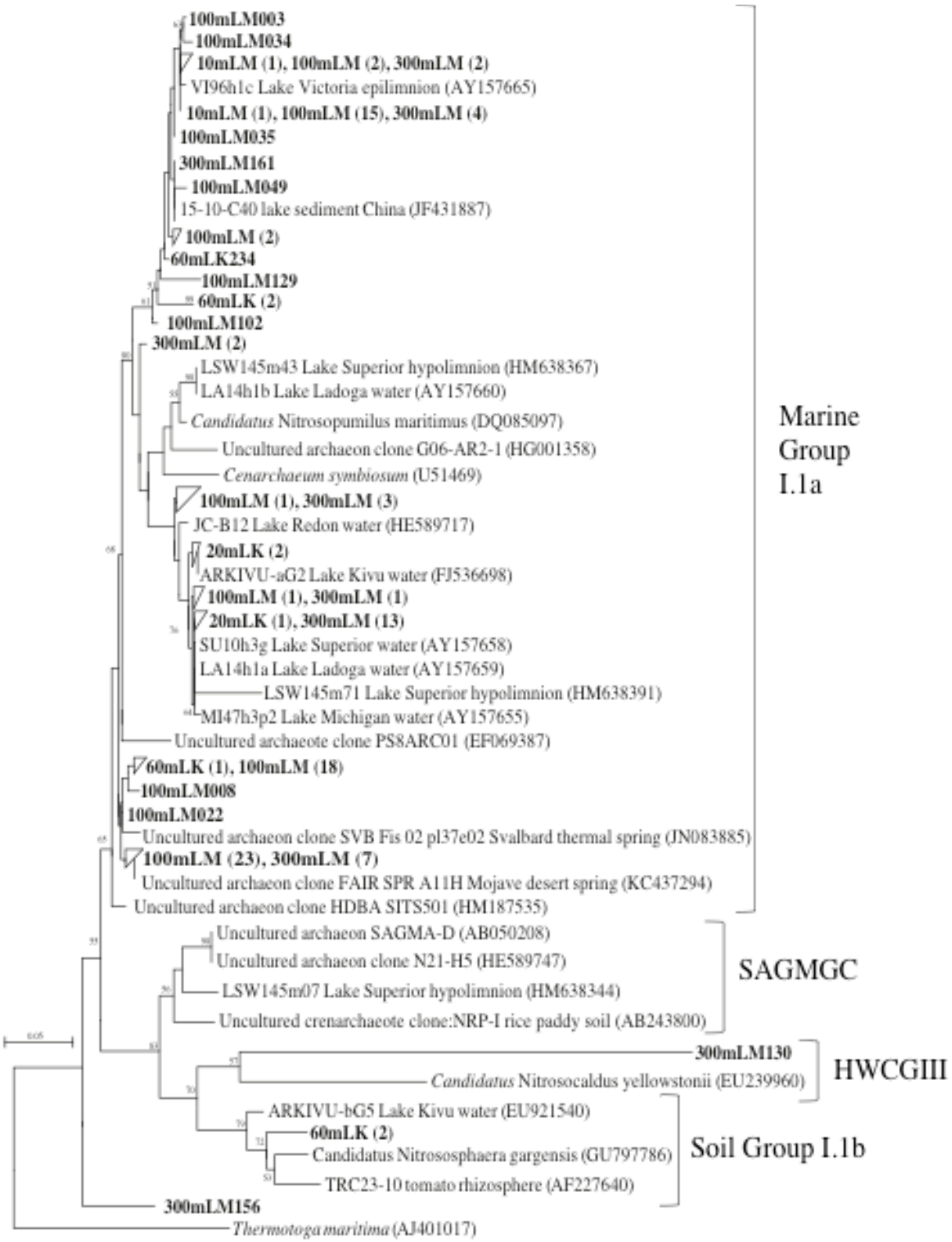
100mLM003
100mLM034
10mLM (1), 100mLM (2), 300mLM (2)
VI96h1c Lake Victoria epilimnion (AY157665)
10mLM (1), 100mLM (15), 300mLM (4)
100mLM035
300mLM161
100mLM049
15-10-C40 lake sediment China (JF431887)
100mLM (2)
60mLK234
100mLM129
60mLK (2)
100mLM102
300mLM (2)
LSW145m43 Lake Superior hypolimnion (HM638367)
LA14h1b Lake Ladoga water (AY157660)
Candidatus Nitrosopumilus maritimus (DQ085097)
Uncultured archaeon clone G06-AR2-1 (HG001358)
Cenarchaeum symbiosum (U51469)
100mLM (1), 300mLM (3)
JC-B12 Lake Redon water (HE589717)
20mLK (2)
ARKIVU-aG2 Lake Kivu water (FJ536698)
100mLM (1), 300mLM (1)
20mLK (1), 300mLM (13)
SU10h3g Lake Superior water (AY157658)
LA14h1a Lake Ladoga water (AY157659)
LSW145m71 Lake Superior hypolimnion (HM638391)
MI47h3p2 Lake Michigan water (AY157655)
Uncultured archaeote clone PS8ARC01 (EF069387)
60mLK (1), 100mLM (18)
100mLM008
100mLM022
Uncultured archaeon clone SVB Fis 02 pl37e02 Svalbard thermal spring (JN083885)
100mLM (23), 300mLM (7)
Uncultured archaeon clone FAIR SPR A11H Mojave desert spring (KC437294)
Uncultured archaeon clone HDBA SITS501 (HM187535)
Marine Group I.1a
Uncultured archaeon SAGMA-D (AB050208)
Uncultured archaeon clone N21-H5 (HE589747)
LSW145m07 Lake Superior hypolimnion (HM638344)
Uncultured crenarchaeote clone:NRP-I rice paddy soil (AB243800)
SAGMGC
300mLM130
Candidatus Nitrosocaldus yellowstonii (EU239960)
HWCGIII
ARKIVU-bG5 Lake Kivu water (EU921540)
60mLK (2)
Candidatus Nitrososphaera gargensis (GU797786)
TRC23-10 tomato rhizosphere (AF227640)
Soil Group I.1b
300mLM156
Thermotoga maritima (AJ401017)

Fig. 6A. Neighbor-joining phylogenetic tree of thaumarchaeotal clones from Lakes Malawi and Kivu based on partial 16S rDNA sequences. Major clades in this phylum are shown at the right. The Marine Group I clade is also known as the group I.1a. Clones from this study are shown in bold typeface. These clones were designated by depth in meters, lake (LM-Lake Malawi, LK-Lake Kivu, and clone number). Accession numbers for 16S rDNA sequences from GenBank are shown in parentheses.

Fewer archaeal sequences were affiliated with the Crenarchaeota than other archaeal phyla, and these sequences were exclusively found in anoxic portions of the hypolimnia of Lakes Kivu (60 m) and Malawi (300 m; Fig. 6B). A few of these sequences fell within Miscellaneous Crenarchaeotal Groups, but most sequences (primarily from Lake Kivu) fell within a cluster closely related to the Group 1.2 C3 Crenarchaeota, which contains sequences recovered from both marine and freshwater environments across the world. There was a diverse group of euryarchaeotal sequences (Fig. 6C) primarily from deeper areas of Lakes Kivu (60 m) and Malawi (300 m) that were affiliated with the Rice Cluster-V (RC-V), Deep Sea Euryarchaeotal Group (DSEG), and Lake Dagow Sediment (LDS) groups.

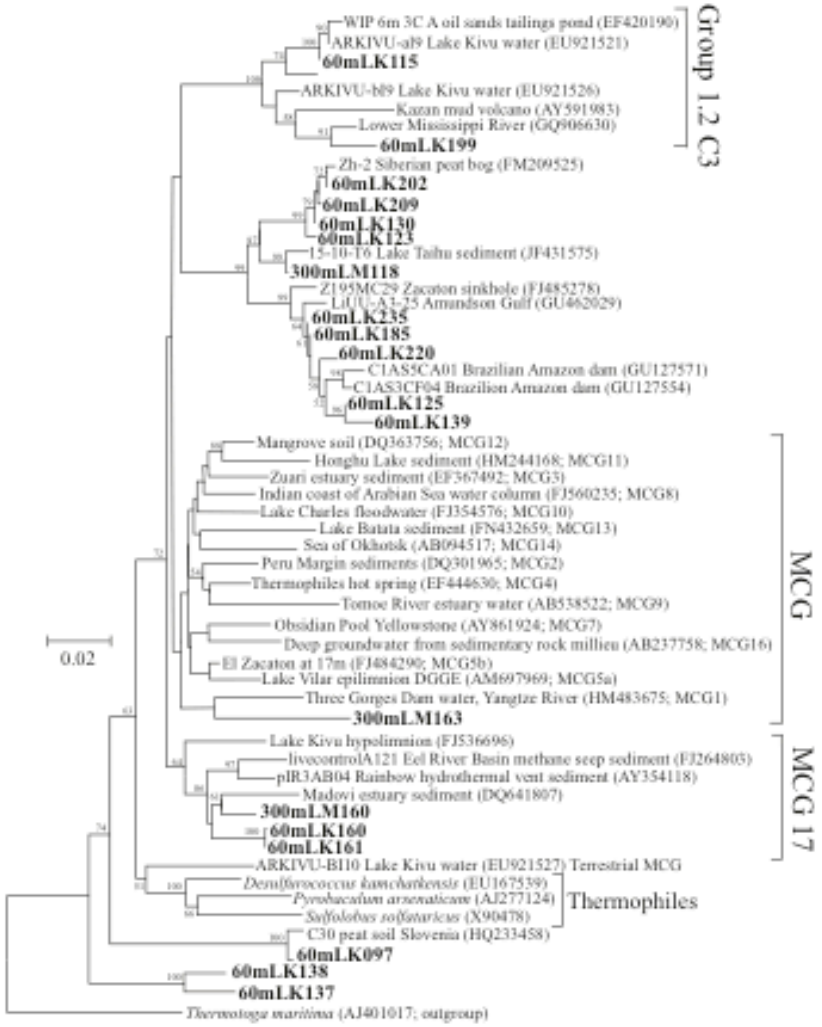
WIP 6m 3C A oil sands tailings pond (EF420190)
ARKIVU-al9 Lake Kivu water (EU921521)
60mLK115
ARKIVU-b89 Lake Kivu water (EU921526)
Kazan mud volcano (AY591983)
Lower Mississippi River (GQ906630)
60mLK199
Group 1.2 C3
Zh-2 Siberian peat bog (FM209525)
60mLK202
60mLK209
60mLK130
60mLK123
15-10-T6 Lake Taihu sediment (JF431575)
300mLM118
Z195MC29 Zacaton sinkhole (FJ485278)
LiUU-A1-25 Amundson Gulf (GU462029)
60mLK235
60mLK185
60mLK220
C1AS5CA01 Brazilian Amazon dam (GU127571)
C1AS3CF04 Brazilion Amazon dam (GU127554)
60mLK125
60mLK139
Mangrove soil (DQ363756; MCG12)
Honghu Lake sediment (HM244168; MCG11)
Zuari estuary sediment (EF367492; MCG3)
Indian coast of Arabian Sea water column (FJ560235; MCG8)
Lake Charles floodwater (FJ354576; MCG10)
Lake Batata sediment (FN432659; MCG13)
Sea of Okhotsk (AB094517; MCG14)
Peru Margin sediments (DQ301965; MCG2)
Thermophiles hot spring (EF444630; MCG4)
Tomoe River estuary water (AB538522; MCG9)
Obsidian Pool Yellowstone (AY861924; MCG7)
Deep groundwater from sedimentary rock millieu (AB237758; MCG16)
El Zacaton at 17m (FJ484290; MCG5b)
Lake Vilar epilimnion DGGE (AM697969; MCG5a)
Three Gorges Dam water, Yangtze River (HM483675; MCG1)
300mLM163
MCG
Lake Kivu hypolimnion (FJ536696)
livecontrolA121 Eel River Basin methane seep sediment (FJ264805)
pIR3AB04 Rainbow hydrothermal vent sediment (AY354118)
Madovi estuary sediment (DQ641807)
300mLM160
60mLK160
60mLK161
MCG 17
ARKIVU-BI10 Lake Kivu water (EU921527) Terrestrial MCG
Desulfurococcus kamchatkensis (EU167539)
Pyrobaculum arsenaticum (AJ277124)
Sulfolobus solfataricus (X90478)
Thermophiles
C30 peat soil Slovenia (HQ233458)
60mLK097
60mLK138
60mLK137
Thermotoga maritima (AJ401017; outgroup)
0.02

Fig. 6B. Neighbor-joining phylogenetic tree of crenarchaeotal clones from Lakes Malawi and Kivu based on partial 16S rDNA sequences. Some clades in this phylum are shown at the right, including the miscellaneous crenarchaeotal groups (MCG). Clones from this study are shown in bold typeface. These clones were designated by depth in meters, lake (LM-Lake Malawi LK-Lake Kivu), and a clone number). Accession numbers for 16S rDNA sequences from GenBank are shown in parentheses.

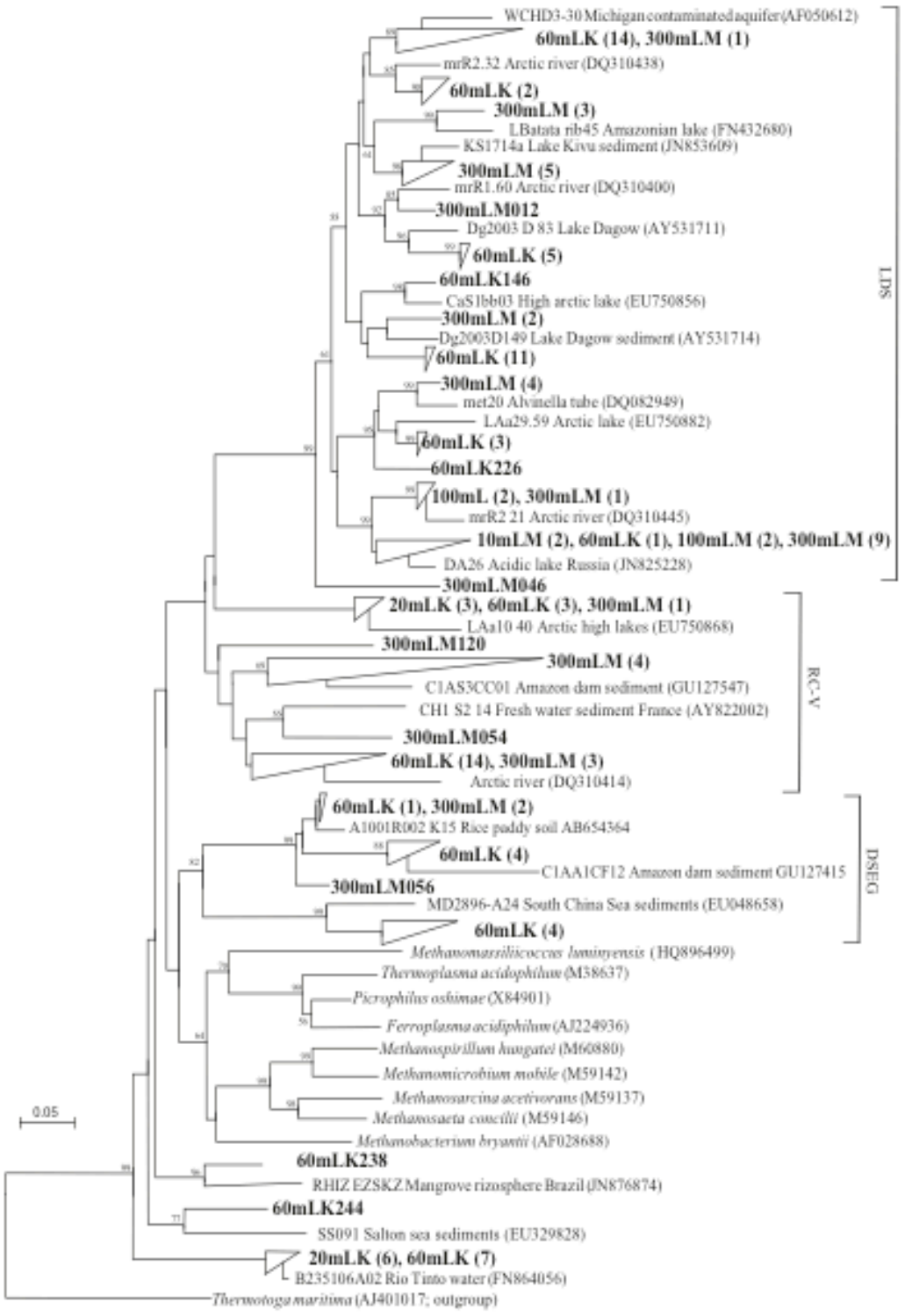
WCHD3-30 Michigan contaminated aquifer (AF050612)
60mLK (14), 300mLM (1)
mrR2.32 Arctic river (DQ310438)
60mLK (2)
300mLM (3)
LBatata rib45 Amazonian lake (FN432680)
KS1714a Lake Kivu sediment (JN853609)
300mLM (5)
mrR1.60 Arctic river (DQ310400)
300mLM012
Dg2003 D 83 Lake Dagow (AY531711)
60mLK (5)
60mLK146
CaS1bb03 High arctic lake (EU750856)
300mLM (2)
Dg2003D149 Lake Dagow sediment (AY531714)
60mLK (11)
300mLM (4)
met20 Alvinella tube (DQ082949)
LAa29.59 Arctic lake (EU750882)
60mLK (3)
60mLK226
100mL (2), 300mLM (1)
mrR2 21 Arctic river (DQ310445)
10mLM (2), 60mLK (1), 100mLM (2), 300mLM (9)
DA26 Acidic lake Russia (JN825228)
300mLM046
20mLK (3), 60mLK (3), 300mLM (1)
LAa10 40 Arctic high lakes (EU750868)
300mLM120
300mLM (4)
C1AS3CC01 Amazon dam sediment (GU127547)
CH1 S2 14 Fresh water sediment France (AY822002)
300mLM054
60mLK (14), 300mLM (3)
Arctic river (DQ310414)
60mLK (1), 300mLM (2)
A1001R002 K15 Rice paddy soil AB654364
60mLK (4)
C1AA1CF12 Amazon dam sediment GU127415
300mLM056
MD2896-A24 South China Sea sediments (EU048658)
60mLK (4)
Methanomassiliicoccus luminyensis (HQ896499)
Thermoplasma acidophilum (M38637)
Picrophilus oshimae (X84901)
Ferroplasma acidiphilum (AJ224936)
Methanospirillum hungatei (M60880)
Methanomicrobium mobile (M59142)
Methanosarcina acetivorans (M59137)
Methanosaeta concilii (M59146)
Methanobacterium bryantii (AF028688)
60mLK238
RHIZ EZSKZ Mangrove rizosphere Brazil (JN876874)
60mLK244
SS091 Salton sea sediments (EU329828)
20mLK (6), 60mLK (7)
B235106A02 Rio Tinto water (FN864056)
Thermotoga maritima (AJ401017; outgroup)
0.05
LDS
RC-V
DSEG

Fig. 6C. Neighbor-joining phylogenetic tree of euryarchaeotal clones from Lakes Malawi and Kivu based on partial 16S rDNA sequences. Major clades in this phylum are shown at the right (LDS – Lake Dagow Sediment; RC-V – Rice Cluster V; DSEG – Deep Sea Euryarchaeotal Group). Clones from this study are shown in bold typeface. These clones were designated by depth in meters, lake (LM-Lake Malawi, LK-Lake Kivu), and a clone number). Accession numbers for 16S rDNA sequences from GenBank are shown in parentheses.

Discussion

Archaea are increasingly being considered important players in the nitrogen and carbon cycles of oligotrophic lakes, but much remains unexplored. The gene abundance and DNA sequence data presented here suggest that planktonic *Archaea* are more prevalent in the hypolimnia of Lake Malawi and Kivu, and might play an important role in nitrogen cycling in these lakes.

The abundance of total *Archaea* increased about two orders of magnitude from the epilimnion (~10^4 16S rRNA gene copies/L) to the deeper hypolimnion (~10^6 gene copies/L) in Lakes Malawi and Kivu during the period of thermal stratification. Archaeal abundance was also evaluated at the same sites in Lake Malawi during 2010 by measuring concentrations of intact and core isoprenoid glycerol dialkyl glycerol tetrather (GDGT) lipids, characteristic of the Thaumarchaeota (Woltering, 2011). A similar pattern of higher GDGT concentrations and archaeal gene markers in the hypoliminon was observed at these sites (NB, CB, and SB), but the GDGT lipids decreased in concentration in the anoxic portion of the hypolimina at two sites (NB and CB) unlike the archaeal gene markers. (Fig. 7). Although the intact GDGTs usually peaked in abundance just below the deep chlorophyll layer around 50 m, the core GDGT lipid concentrations increased at 100 m before decreasing at deeper depths. This type of change in planktonic archaeal abundance has also been observed before in both ocean (Massana et al., 2000, Karner et al., 2001) and lake environments (Hongchen et al., 2009). Kish (2010) observed a similar sharp decrease in total archaeal abundance during thermally stratified conditions in Lake Superior, a

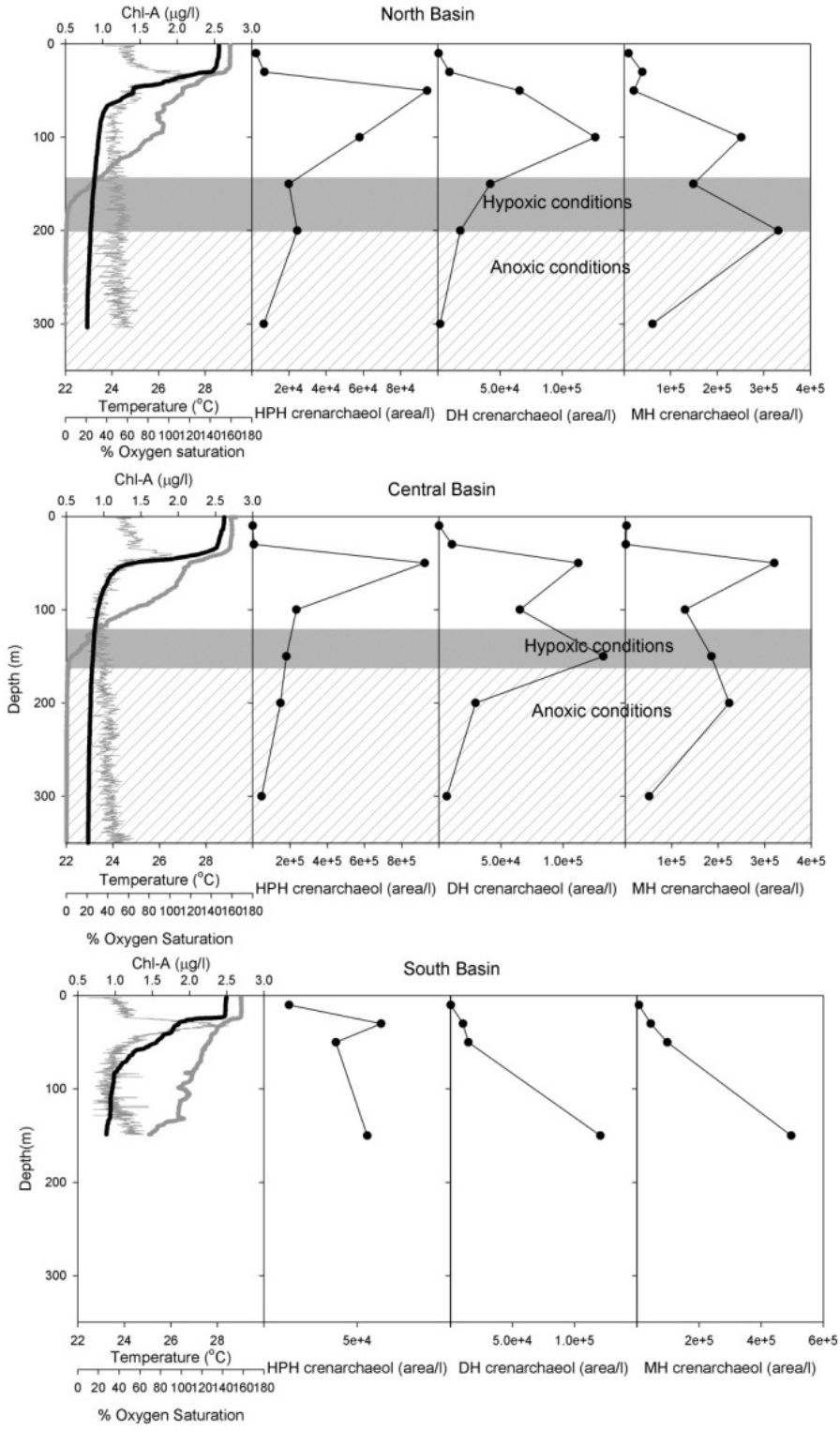
North Basin
Chl-A (µg/l)
Hypoxic conditions
Anoxic conditions
Temperature (°C)
% Oxygen saturation
HPH crenarchaeol (area/l)
DH crenarchaeol (area/l)
MH crenarchaeol (area/l)
Central Basin
Depth (m)
% Oxygen Saturation
South Basin
Depth(m)

Fig. 7. Vertical profiles of intact isoprenoid GDGT lipids in suspended particular matter (SPM) relative to temperature, and oxygen and chlorophyll concentrations in the water column at the North, Central and South Basins of Lake Malawi in January 2010. From Woltering, 2011.

temperate oligotrophic great lake in North America. During mixed lake conditions in May and July, archaeal abundance remained relatively stable with depth in Lake Superior, averaging about 10^6 gene copies/L. Archaeal abundance decreased about two orders of magnitude in the warm epilimnetic waters after the water column became thermally stratified in August and September, and the composition of the epilimnetic archaeal community changed. He associated these changes more with the seasonal development of the thermocline rather than the influence of a deep chlorophyll maximum.

The sharp increase in the abundance of total *Archaea* below the thermocline in Lakes Malawi and Kivu suggests that some parameter may limit their productivity and abundance in surface waters. The abundance of planktonic *Archaea* might be controlled by temperature, water quality, or light. In a previous study of planktonic *Archaea* in the North Sea, temperature was negatively correlated with the abundance of the MG-I *Archaea* (Herfort et al., 2007). In a recent publication, Vissers et al. (2013) reported an inverse correlation between the abundance of Thaumarchaeota and both temperature and salinity in Lake Lucerne. This situation could explain the observed low AOA abundance in surface waters and the increase in AOA abundance below the deep chlorophyll layer in Lake Malawi. However, water temperature may not be the driving force in these tropical great lakes because it was not correlated with the abundance of MGI or the total Archaea ($p=0.16$ and 0.06, respectively). Only the distribution of the *amo*A revealed a slight correlation with temperature across all sites in Lake Malawi (Pearson correlation, $p<0.01$, $r^2=-0.35$) where the highest abundances were seen in the cooler water. However,

it is hard to interpret the influence of temperature alone on abundance from these data alone because of the lack of samples from the mixed period in Lake Malawi.

Water quality may also control the distribution and diversity of planktonic archaeal populations and communities. Auguet and Casamayor (2013) found that the MG-I and SAGMGC-1 members of the Thaumarchaeota had their highest relative abundances in the most oligotrophic of the various high mountain lakes they investigated in the Pyrenees of northeast Spain. They suggested that pH was the main environmental factor controlling the composition of planktonic archaeal communities in these lakes. In another investigation, Auguet et al. (2011) found lower numbers of these organisms in eutrophic Lake Vilar and no correlation with measured environmental parameters.

It is well documented that ammonia oxidizing *Archaea* (AOA) are more abundant than ammonia oxidizing bacteria in groundwater (Reed et al., 2010), and in the water column of the open ocean (Wuchter et al., 2006, Mincer et al., 2007, Bouskill et al., 2012) as well as suboxic basins (Coolen et al., 2007, Lam et al., 2007). AOA can more efficiently use low concentrations of ammonia than ammonia oxidizing bacteria (AOB; Martens-Habbena et al., 2009). Sims and collaborators (2012) found AOA to be an order of magnitude more abundant than AOB in low-ammonium wetlands, suggesting low concentrations of NH_4^+, O_2, and organic matter granted the AOA a competitive advantage over the AOB. Similarly, Konneke et al., (2005) found that low concentrations of organic compounds appeared to inhibit the growth and ammonia oxidation of *Nitrosopumilus maritimus*. Thus, AOA might be outcompeted by heterotrophic microbes and small-size phytoplankton for available ammonia in the

euphotic zone, especially when dissolved organic carbon is high (Herfort et al., 2007, French et al., 2012, Small et al., 2013).

Bootsma and Hecky (1999) published N species profiles in Lake Malawi where it is clear that nitrate is high in the oxic hypolimnion and very low in the epilimnion (Fig. 8). Likewise, nutrient profiles for Lake Kivu show an increase of ammonium beneath the oxic-anoxic boundary, along with a slight increase in nitrate (Fig. 9). This observation supports the contention that AOA, which oxidize ammonia, may survive well and be detected in the hypolimnia of these lakes. In Lake Malawi, the strongly stratified dry period (September to December) has been described as a low productivity period, while the wet stratified season (January to May) when most samples were taken in this study sees the highest photosynthetic rates (Guildford and Taylor, 2011). The decrease in planktonic archaeal and *amo*A abundances I observed in the epilimnion could therefore be driven by competition with phytoplankton during the high photosynthetic productivity period during the wet season.

Light can also inhibit archaeal ammonia-oxidizers more than their bacterial counterparts in laboratory enrichment cultures, which could further explain the lower abundance of AOA in the euphotic zone of the water column (Merbt et al., 2012). Konneke et al. (2005) were the first to demonstrate that an isolated species of the Marine Group I.1a Crenarchaeota (now Thaumarchaeota) was capable of oxidizing ammonia. Since then, several other uncultivated clades, including the soil Group I.1b, SAGMGC-1, and HWCGIII clusters, have been demonstrated to possess an archaeal form of the α-

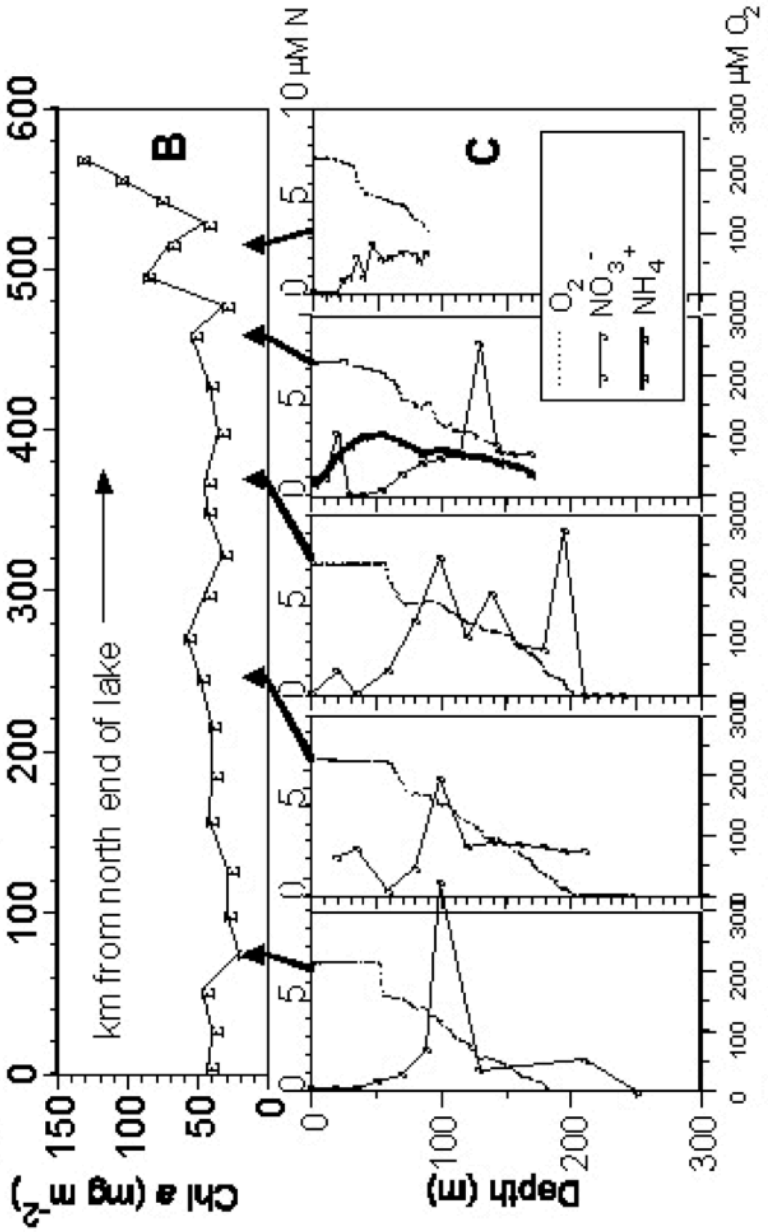
B
C
km from north end of lake
Chl a (mg m-2)
Depth (m)
10 µM N
µM O2
O2
NO3+
NH4

Fig. 8. Profiles of chlorophyll, oxygen and nitrogen species across the length of Lake Malawi. From Bootsma and Hecky (1999).

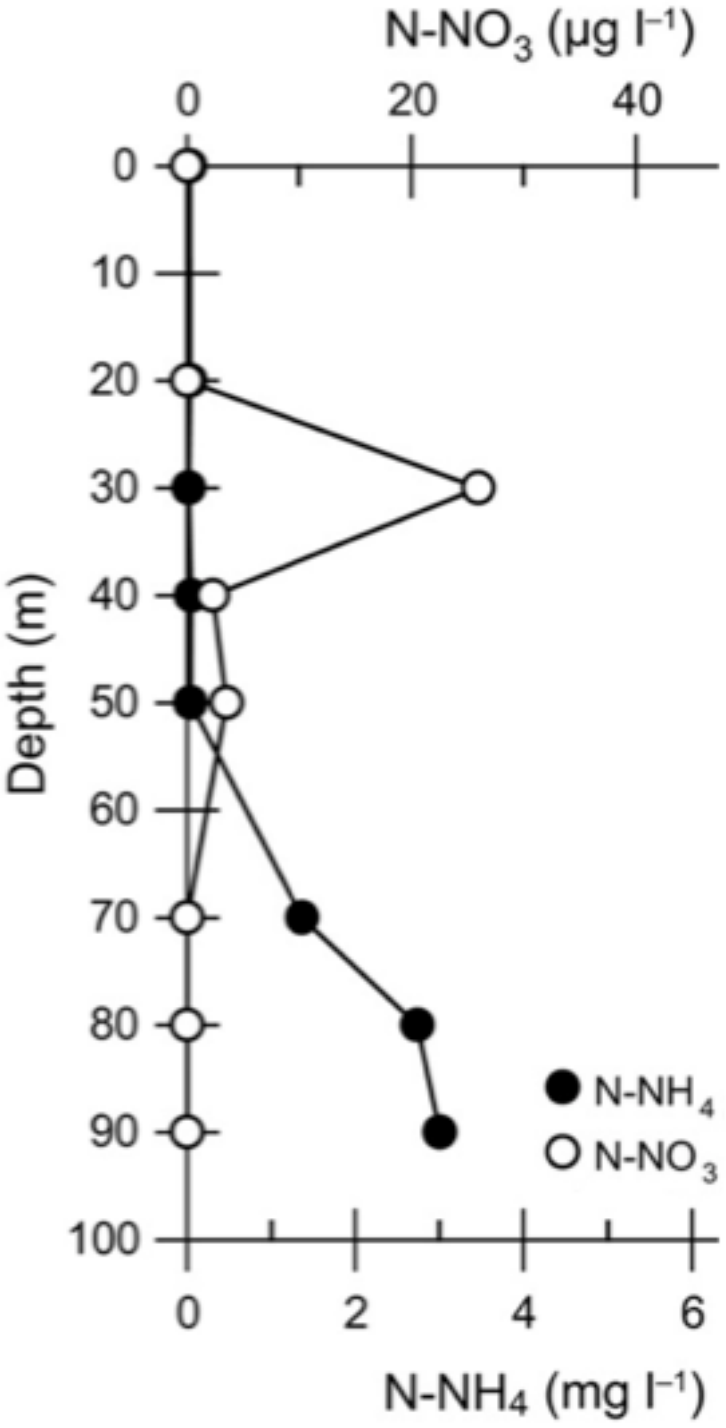
N-NO3 (µg l-1)
0
20
40
0
10
20
30
40
50
60
70
80
90
100
Depth (m)
N-NH4
N-NO3
0
2
4
6
N-NH4 (mg l-1)

Fig. 9. Profiles of ammonia and nitrate for a site in the Eastern Basin in Lake Kivu, close to the basin sampled in this study. Modified from Lliros et al., (2010).

subunit of the ammonia monooxygenase (*amo*A) gene (Pester et al., 2011). A one-to-one relationship between the archaeal *amo*A and 16S rRNA gene abundances has been observed in some oligotrophic marine and lake ecosystems (Church et al., 2010, Vissers et al., 2013). In this study, the archaeal *amo*A gene abundance was larger than the total *Archaea* and MG-I abundances in most cases in the deep hypolimnion (ranging from 3 to 42 times more abundant than the 16S rDNA). Excluding surface water samples, the MG-I distribution was correlated with the archaeal *amo*A gene abundance at all sites ($r^2 = 0.72$, $p < 0.01$) and this relationship had a slope of 3.47. A similar relationship was observed in Lake Superior (Hicks et al., unpublished data), various oligotrophic ocean sites (Mincer et al., 2007, Santoro et al., 2010), and 3:1 ratio was reported in two studies of planktonic *Archaea* in the North Sea (Wuchter et al., 2006, Herfort et al., 2007). This high archaeal *amo*A:16S rRNA gene ratio could be interpreted as the number of copies of the *amo*A gene that each thuamarchaeal cell contains. It is important to note that qPCR data are not necessarily comparable between different target sequences because of biases inherent to any PCR reaction, the different efficiency of each reaction, and the substantial difference in fragment size (Suzuki and Giovannoni 1996, Freeman et al., 1999). Thus, earlier studies have attributed unusual ratios of archaeal *amo*A:16S rDNA to primer bias and paucity of specificity in the primer set (Konstantinidis et al., 2009, Church et al., 2010).

It was interesting that the archaeal *amo*A gene abundance remained high and sometimes peaked in suboxic or anoxic waters at most sites in Lake Malawi (Fig. 3). Although archaeal ammonia-oxidation is an aerobic process, it has been found to occur

optimally at low oxygen concentrations (Martens-Habbena et al., 2009, Pitcher et al., 2011). This fact could help explain why the archaeal 16S rDNA and *amo*A gene abundances remained high in suboxic or anoxic waters and peaked in these deeper waters at all sites except SB. The archaeal *amo*A gene has been detected in significant numbers in the oxygen-minimum zones of the Pacific Ocean (Bouskill et al., 2012), Arabian sea (Pitcher et al., 2011), and human-made environments like wastewater treatment plants (Sauder et al., 2012). AOA were found to be abundant at the top and bottom layers of the oxygen minimum zone (OMZ) in the Arabic sea, but their abundance dropped significantly in the core of the OMZ, suggesting a minimum requirement of oxygen between 2.5 and 5 μM (Pitcher et al., 2011). Coolen and collaborators (2007) also found a peak in abundance of archaeal *amo*A and MG-I in the deep, suboxic waters of the Black Sea at around 70 m and 10 μM of dissolved oxygen. Other studies have shown either no effect of dissolved oxygen on the transcript numbers of archaeal *amo*A (Treusch et al., 2005, Abell et al., 2011) or have found it in completely anoxic waters (Treusch et al., 2005). In addition, mRNA for bacterial *amo*A and 16S rRNA has been detected weeks to years after starvation in laboratory cultures (French et al., 2012). This finding suggests that detecting archaeal 16S rRNA and *amo*A genes in the anoxic waters of Lake Malawi may simply be due to inactive cells sinking while attached to particles from the upper water column.

A major portion of Thaumarchaeota sequences from several depths in Lakes Malawi and Kivu clustered within the Marine Group I.1a as expected (Fig. 6A). Kish (2010) primariy found MG-I clones in shallow (30 m) and deep (145 m) water, and surficial

sediments of Lake Superior. Unlike Lake Superior, the MG-I might not be the primary archaeal clade in the water column of Lake Malawi because the number of qPCR gene copies from this clade was usually less than that for the total *Archaea* at most sites in Lake Malawi (Fig. 3). In addition, two sequences were affiliated with the Group I.1b (soil) and one in the Hot Water Crenarchaeotic group III (HWCGIII), initially found associated to hydrothermal vents and hot springs.

Interestingly, none of the archaeal 16S rRNA sequences from this study were affiliated with other Thaumarcheotal clades such as the pSL12, ALOHA, and Group I.1c clusters. Some members of the pSL12 clade may contain an archaeal *amo*A-like gene (Mincer et al., 2007). This group has been found in the surface and deep waters of some parts of the Chesapeake Bay and the Sargasso Sea (Bouskill et al., 2012). The SAGMGC-I, another freshwater clade of Thaumarchaeota that contains the *amo*A gene, can be more abundant than the MG-I in both acidic and neutral, high-mountain, oligotrophic lakes, and have not been found in marine waters (Auguet and Casamayor, 2008; Auguet and Casamayor, 2013). None of our sequences were associated with this group.

The Crenarchaeota represented the smallest fraction of archaeal sequences recovered, with only 19 clonal sequences recovered from the anoxic water samples of Lake Malawi (300 m) and Lake Kivu (60 m; Fig. 6B). Kubo and collaborators (2012) recently identified an environmentally widespread and genetically diverse group of Crenarchaeota that they classified into 17 subgroups, collectively refered to as the Miscellaneous Crenarchaeotal Group, of which little else is known. All except three

sequences recovered from Lakes Malawi and Kivu fell within Miscellaneous Crenarchaeotal Groups, alongside sequences from very diverse freshwater environments, which suggests no common biogeography.

Besides hydrothermal vents, freshwater ecosystems might be expected to harbor the greatest archaeal diversity (Auguet et al., 2010). A large number (92) and proportion (43%) of all archaeal sequences in this study, primarily from the deepest depths sampled in Lake Malawi (300 m) and Lake Kivu (60 m), were affiliated with uncultivated euryarchaeotal clades. These 16S rRNA gene sequences were affiliated with the Euryarchaeotal Rice Cluster V (RC-V; Grosskopf et al., 1998), Lake Dagow Sediment (LDS), and Deep Sea Euryarchaeotal Group (DSEG) clusters (Fig. 6C). Sequences from these groups have been found in various freshwater environments, both oxic and anoxic, and might be significantly more diverse than the ubiquitous and more studied *Thaumarchaeota* (Auguet et al., 2010, Barberan et al., 2011). Sequences from the RC-V cluster, a group whose functions remain unknown (Pouliot et al., 2009), have been found previously in Lake Kivu surface sediments (Bhatterai et al., 2012), The RC-V cluster is phylogenetically diverse and sequences from this group have been reported in a large variety of environments (Galand et al., 2006). In addition to the RC-V cluster, little is known about the physiology of the other uncultivated euryarchaeotal clades. Although some functional genes have been found in uncultured marine Euryarchaeota that might explain their ecological roles (Iverson et al., 2012), there is still no satisfactory explanation for the abundant and widespread mesophillic members of this phylum in oxic freshwater (Barberan et al., 2011).

Archaea belonging to the methanogenic Euryarchaeota are commonly found in anoxic freshwater environments like sediments (Hershberger et al., 1996, Schleper et al., 1997) and deep the anoxic water column of deep lakes (Lliros et al., 2008) or wetlands (Fedotova et al., 2012). Surprisingly, none of the sequences recovered in this study were affilated with any known methanogenic archaeal taxa, even though they were isolated at a water depth (60 m) below the oxic/anoxic boundary in Lake Kivu (Schmid et al., 2005). DNA sequences from two lineages of methanogenic *Archaea* (Methanosarcinales and Methanocellales) have been recovered previously from primarily anoxic waters of this lake (Lliros et al., 2010). Interestingly, Bhattarai et al., (2012) recently indicated that DNA sequences from methanogens were surprisingly absent or rare in the surface sediments of Lake Kivu even though a large quantity of methane of biological origin is dissolved in the hypolimnion of this lake.

This study provides the first examination of archaeal abundance and diversity in Lake Malawi and the second known description of this microbial domain in the water column of Lake Kivu. Similar to Lake Superior, the abundance of archaeal groups estimated by a culture-independent qPCR approach indicated that total and MG-I *Archaea* were approximately two orders of magnitude less abundant in the warmer epliminion compared to the deeper and cooler hypolimnetic waters of both Lakes Malawi and Kivu, which may explain why fewer archaeal clones were obtained from the epilimnion of these lakes. Unlike Lake Superior, members of several other uncultivated thaumarchaeota besides the MG-I appear to be present in these lakes. This assertion was supported both by differences in the abundance of

total and MG-I specific 16S rRNA genes and archaeal 16S rDNA sequences obtained from these lakes. In addition, 16S rDNA sequences from the Miscellaneous Crenarchaeotal Group and several uncultivated euryarchaeotal clades indicate a broad and cosmopolitan diversity of archaeal groups. Future investigations should evaluate seasonal changes in planktonic archaeal abundance and diversity in Lakes Malawi and Kivu as well as their potential role in nitrification compared to bacteria.

CHAPTER III
Spatial variations in the abundance of *Archaea* in Lake Superior

Introduction

Lake Superior is arguably the most oligotrophic great lake in the world (Gildford et al., 2000), yet its hypolimnion has been experiencing an increase in NO_3^- during the last 60 years (Sterner et al., 2007). It is widely believed that algal blooms are being kept in check by the severe P limitation in the water column, and there is real concern for the possible consequences of increasing nutrient loads (Sterner et al., 2004). However, recent studies suggest that NO_3^- increases are probably autochtonous and the product of biological nitrification (Finlay et al., 2007).

The microbial loop is frequently overlooked in food web modeling (Hudson et al., 2005). A recent study showed that bacterioplankton in Lake Superior are important in accumulating and breaking down polychlorinated biphenyls and other persistent bioaccumulative toxic organic contaminants, more so than pythoplankton (Hudson et al., 2005). The increase of nutrient inputs into sensitive ecosystems, and the potential for transport of these nutrients, may affect microbial communities and ultimately alter biogeochemical cycling (Cotner and Biddanda 2002). However, the exact composition of the microbial loop has been greatly overlooked in oligotrophic great lakes, and particularly in Lake Superior (Reed and Hicks 2010).

It is now widely accepted that mesophilic Marine Group I (MG-I) *Archaea* in the Thaumarchaeota phylum are active nitrifiers that carry out the rate-limiting step in the removal of nitrogen from aquatic systems, namely ammonia oxidation (Lliros

et al., 2010, Walker et al., 2010, Small et al., 2013). All organisms in this group have been found to possess a copy of an archaeal ammonia monooxygenase α subunit gene (*amo*A), which has so far not been found in organisms that cannot perform ammonia oxidation in the laboratory (Pester et al., 2011). Kish (2010) did a high-resolution study on archaeal abundance at one site in western Lake Superior from early summer to late fall. He found that *Archaea* thrive in the cooler waters below the thermocline during the stratified summer, but remain at high abundance throughout the water column during the mixed period in winter. He also found a distinct community formed in the surface during the stratified period, significantly different from the archaeal community in the hypolimnion. This study compares the distribution of total *Archaea*, the MG-I and the archaeal *amo*A gene in the epilimnion and hypolimnion at different sites across Lake Superior in late summer in an attempt to confirm whether the vertical pattern of archaeal abundance seen by Kish (2010) is common across this lake.

Methods

Field Site Locations and Sampling

To complement Kish's (2010) work at one site in western Lake Superior, seven sites across Lake Superior were sampled at two depths within the epilimnion and hypolimnion during the late summer of 2009 and 2010 (Fig. 10). This sampling pattern allowed for a spatial comparison of archaeal abundance during the stratified period in September and October in this lake (Table 5). Lake water was collected from the epilimnion (typically 5 m) and the hypolimnion (100 m) during two cruises

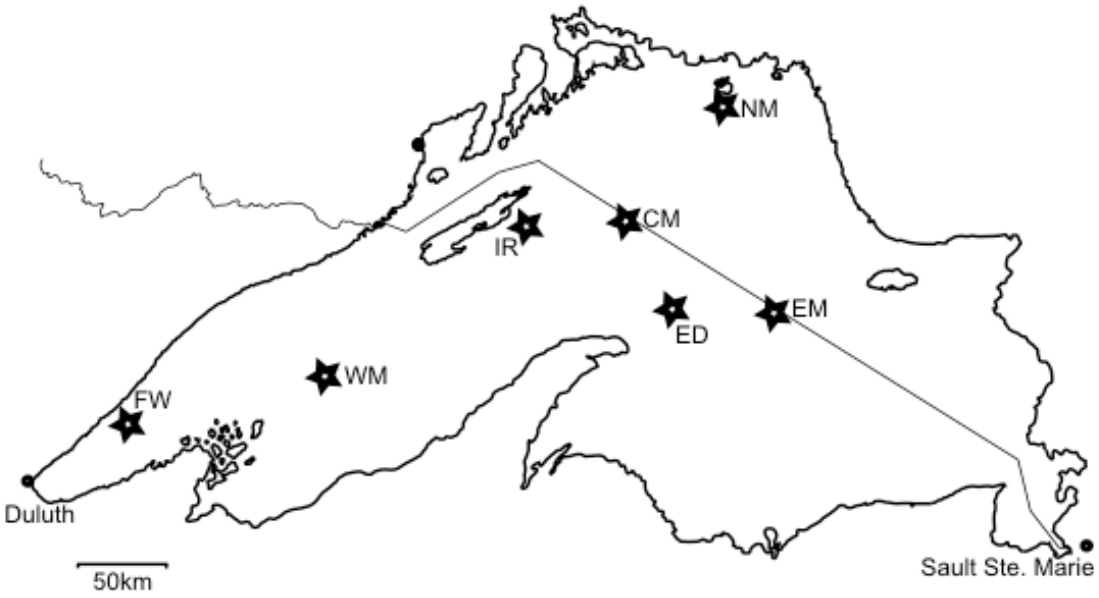
NM
CM
IR
EM
ED
WM
FW
Duluth
50km
Sault Ste. Marie

Fig. 10. Locations of sampling sites in Lake Superior for the 2009 and 2010 stratified season.

from October 4-6, 2009, and September 21-25, 2010 onboard the *R/V Blue Heron* using a rosette sampler containing a CTD and 8L Niskin bottles (Table 5).

Prokaryotic Cell Abundance

Triplicate subsamples (10 ml) of water were taken for estimating prokaryotic cell abundance and preserved with 0.5 ml of 37% paraformaldehyde (~2% final concentration). They were

stored at 4°C for a maximum of 2 weeks before counting prokaryotic cells by epifluorescence microscopy according to Porter and Feig (1980). Microorganisms were collected by filtering 2 ml of preserved water sample onto a black polycarbonate filter (25 mm diameter, 0.22 μm pore size, GE Water & Process Technologies) and stained with 100 μL of 10 μM DAPI.

DNA Extraction

A larger portion of water (7 to 60 L) from each depth was pressure filtered on board through Durapore membrane filters (142 mm diameter, 0.22 μm pore size, Millipore Corporation) no longer than 2 h after collection, until the filter was clogged. The filters were placed in Whirlpak bags, stored at -20ºC, and transported to the lab in a cooler where they were stored at -80ºC for no longer than a month before DNA was extracted.

Total DNA was isolated using a PowerSoil DNA Isolation kit (MoBio Laboratories, Inc.) following the manufacturer's protocol. The large filters were cut into the smallest possible pieces over aluminum foil and 12.5% of the total filter and sample (by weight) was transferred into the initial extraction tube. DNA concentrations

Table 5. Locations in Lake Superior where water samples were collected from the epilimnion and hypolimnion in October 2009 and September 2010.

Site*	Latitude Longitude	Year	Month	Day	Depth (m)	Temp. (ºC)	No. of filters
EM	47º33.37'N 86º35.68'W	2009	October	6	2	9.4	2
					100	4.0	2
		2010	September	22	5	12.5	3
					35	12.2	2
					100	4.0	3
ED	47º31.81'N 87º07.49'W	2010	September	22	5	12.8	2
					100	3.9	1
CM	48°02.66'N 87º47.17'W	2009	October	5	2	8.6	2
					100	3.9	2
		2010	September	22	5	11.4	2
					100	3.8	2
NM	48°41.00'N 86°57.20'W	2009	October	5	2	8.3	2
					100	4.0	2
		2010	September	NA	100	NA	1
IR	47º58.41'N 88º28.08'W	2010	September	22	5	11.4	2
					100	3.8	1
WM	47º19.05'N 89º50.76'W	2009	October	4	10	10.6	2
					100	3.8	2
		2010	September	25	5	8.4	2
					100	3.8	1
FW	47º01.98'N 91º16.50'W	2010	September	21	5	9.3	2
					100	3.8	2

* EM=eastern mooring, ED, CM=central mooring, NM=north mooring, IR=Isle Royale, WM=western mooring, FW=far west, NA= not available

were determined using a NanoDrop 3000 fluorospectrometer (Thermo Scientific Inc.) with Quant-iT Picogreen dye (Invitrogen). DNA extraction efficiency was estimated to be 21% after spiking filters with known concentrations of *E. coli* DNA in three separate extractions.

Abundance of Total Archaea*, Marine Group I* Archaea*, and Ammonia-oxidizing* Archaea

Quantitative polymerase chain reaction (qPCR) assays using Brilliant II SYBR Master Mix (Agilent Technologies) were performed to estimate the abundance of two archaeal groups (*i.e.*, total *Archaea*, Marine Group I *Archaea*) and the archaeal *amo*A gene (see qPCR pimers and

cycling conditions presented in Table 3 in Chapter II). All qPCR reactions were conducted in a Corbett Research Rotor-Gene 3000 thermal cycler. Standards for the qPCR reactions were serial dilutions ranging from 200 pg/μL to 2 ag/μL of gene fragments amplified from PCR products of appropriate Lake Superior clones (Su10h3a referenced in Keough et al., 2004 for total and MG-I *Archaea*, and clone 40m3C3 isolated in R.E. Hicks' lab for the archaeal *amo*A assay). These fragments were initially amplified using the M13 primers supplied with the cloning kits (see below). qPCR reactions were performed in triplicate for each DNA sample, and gene copies were quantified using linear regression and a standard curve relating C_t values to gene copies (typically 10^1 to 10^8 gene copies). No-template control and negative control reactions containing a non-target sequence (*Pseudomonas fluorescens* total DNA) were used to check the specificity of the PCR reactions.

Statistics

The effects of depth on gene abundances were evaluated with a one-way ANOVA with an alpha value of 0.05. Spearman's correlation was performed on the *amo*A gene abundance versus the MG-I and total *Archaea* 16S rDNA abundances, as well as between gene copy numbers and temperature. Where available, samples from the same site were compared between sampling years (2009 and 2010). All analyses were performed using JMP version 7 (SAS Institute Inc.).

Results and Discussion

The water column of Lake Superior was stratified when each site was sampled (Table 5). Temperatures ranged from 3.8-4.0ºC in the hypolimnion (100 m) and 8.3-12.8ºC in the epilimnion (between 2 and 10 m) at sites across the lake. Picoplankton cell abundance was significantly higher ($p < 0.01$), in the epilimnion (range = 1.2-1.9 x 10^6 cells/ml) than in the hypolimnion (range = 0.6-0.8 x 10^6 cells/ml) at all sites (Table 6), which agrees with previous picoplankton abundance measurments estimated during the fall in Lake Superior (Hicks et al., 2004).

Total *Archaea* and MG-I were significantly more abundant ($p = 0.001$ and 0.004 respectively) in the hypolimnion than in the epilimnion at all sites examined, but there was no significant difference in *amo*A gene abundance between the epilimnetic and hypolimnetic samples. No difference between samples from 2009 and 2010 ($p > 0.05$) was detected for total *Archaea*, MG-I *Archaea*, or the archaeal *amo*A gene abundances at the sites that were sampled both years. The abundance of total *Archaea* varied from 0.18 to 1, 368 x 10^4 16S rDNA gene copies/L. This range

of values was larger than the total archaeal abundance that Kish (2010) reported (1.93 to 186 x 10^4 gene copies/L) for planktonic *Archaea* at site WM during thermally stratified condictions in August and September 2007 using the same qPCR assay and primers. However, these values still fall within the range of total archaeal abundance reported elsewhere: Small and collaborators (2013) reported a maximum abundance of 1.9 x 10^7 AOA cells/L at the same site in Lake Superior, althought they were unable to detect them in the surface waters (above 20m). Auguet et al., (2012) detected between 0.2 and 15 x 10^7 archaeal cells/L in the deep, oligotrophic lake Redon during the stratified period. Lliros et al (2010) reported a maximum of around 3 x 10^8 MG-I 16S rRNA gene copies/L in the water column of Lake Kivu.

In both this study and Kish's (2010), total archaeal abundance increased approximately two orders of magnitude from the epilimnion to the hypolimnion. Kish showed this increase often occurred in a stepwise fashion below the thermocline and deep chlorophyll maximum. He attributed this change more to the seasonal development of the thermocline that to the presence of the deep chlorophyll maximum. Lake Superior receives very little irradiance, which may limit phytoplankton production in this lake for almost 8 months of the year (Guildford et al., 2000). Merbt et al., (2012) speculated that light may inhibit archaeal distribution because it may interfer with nitrification. Thus, light in addition to temperature may restrict archaeal growth and abundance during summer months in the surface water of Lake Superior.

Table 6. Abundance of total *Archaea*, Marine Group I *Archaea*, archaeal *amo*A gene, and picoplankton cells at seven sites across Lake Superior during during October 2009 and September 2010. (bdl = below detection limit, nd = not determined, * = × 10^4 gene copies/L, + = × 10^9 cells/L)

Site	Year	Total *Archaea*[*]		Marine Group I[*]		Archaeal *amo*A[*]		Picoplankton Abundance[+]	
		Epi	Hypo	Epi	Hypo	Epi	Hypo	Epi	Hypo
EM	2009	6.41	615	0.59	3.92	0.05	0.92	nd	nd
	2010	4.07	599	0.14	0.69	0.86	62.9	1.78	0.70
ED	2010	0.54	192	bdl	1.77	0.29	1.89	1.88	0.77
CM	2009	76.7	231	0.23	1.58	0.90	5.51	nd	nd
	2010	4.71	471	bdl	0.36	0.44	121	1.46	0.74
NM	2009	4.70	92.6	0.24	4.29	0.04	1.65	nd	nd
	2010	nd	173	nd	0.47	nd	18.3	nd	nd
IR	2010	2.34	667	bdl	0.74	0.05	0.95	1.60	0.69
WM	2009	0.18	331	0.06	1.33	0.66	0.47	nd	nd
	2010	2.96	1,368	bdl	0.71	0.08	3.02	1.23	0.81
FW	2010	6.93	561	bdl	2.02	0.66	9.04	1.40	0.61

The abundance of the MG-I was much lower than that of the total *Archaea* even though the abundance of both groups were correlated ($p < 0.01$) and decreased 1 to 2 orders of magnitude from the epilimnion to hypolimnion (Table 6). Interestingly, MG-I were detected in the surface water at all Lake Superior sties, which was not the case for Lake Malawi (see Chapter II). Small et al., (2013) recently reported a similar increase of archaeal abundance with depth below the thermocline at site WM for an August 2010 cruise. They used CARD-FISH cell counts and the Cren554 probe to estimate crenarchaeotal cell abundance, so their measurements may be more comparable to our qPCR-based MG-I abundances that our total archaeal or archaeal *amo*A gene abundances. Their estimates of crenarchaeotal cells were about three orders of magnitude higher ($\sim 10^6$-10^7 cells/L) than my MG-I abundance measurements ($\sim 10^4$ MG-I 16S rDNA gene copies/L). However, the range of values was similar to the typical range for total archaeal abundance ($\sim 10^6$ total archaeal 16S rDNA gene copies/L) I measured in the hypolimnetic waters of Lake Superior (Table 6).

Interestingly, archaeal *amo*A gene abundance was more strongly correlated ($r^2=0.77$, $p < 0.001$) with total archaeal abundance than with the MG-I archaeal abundance. When all sites were considered together, there was no difference ($p > 0.05$) in the abundance of the archaeal *amo*A gene in the epilimnion and hypolimnion of Lake Superior. However, there were clearly fewer archaeal *amo*A gene copies in the epilimnion verus hypolimnion when individual sites were examined (Table 6). Small et al., (2013) showed a similar pattern for nitrification

rates at site WM in Lake Superior, where the nitrification rate was slow in the epilimnion and faster below 40 m in the hypolimnion. Although ammonia and nitrate were not measured in these samples, it has been seen before that both increase with depth when the water column is stratified, but remain stable when it is mixed (Fig. 11; Ostrom et al., 1998, Kumar et al., 2007, Hicks and Welch, unpublished). It is a possibility that the AOA can take advantage of the ammonia below the euphotic zone, but compete with algae and cyanobacteria for it in the surface water.

There was no significant difference between the abundance of *amo*A in the epi- and hypolimnion at all sites taken together. However, there is a clear difference between sites (most filters were not replicated therefore statistical analyses only represent variation in the qPCR reaction) and taken independently, there is always a higher abundance in the deep water versus the surface. Small et al., (2013) showed AOA abundance peak at around 60m during the stratified period, and nitrification rates showed a step-wise increase at 40m. The sampling depths in this study miss the depths at which these changes take place therefore only detecting the low abundance in the epilimnion, and the dip below the peak. Additionally, their numbers for AOA using CARD-FISH is 3 orders of magnitude higher than the copy numbers in this study.

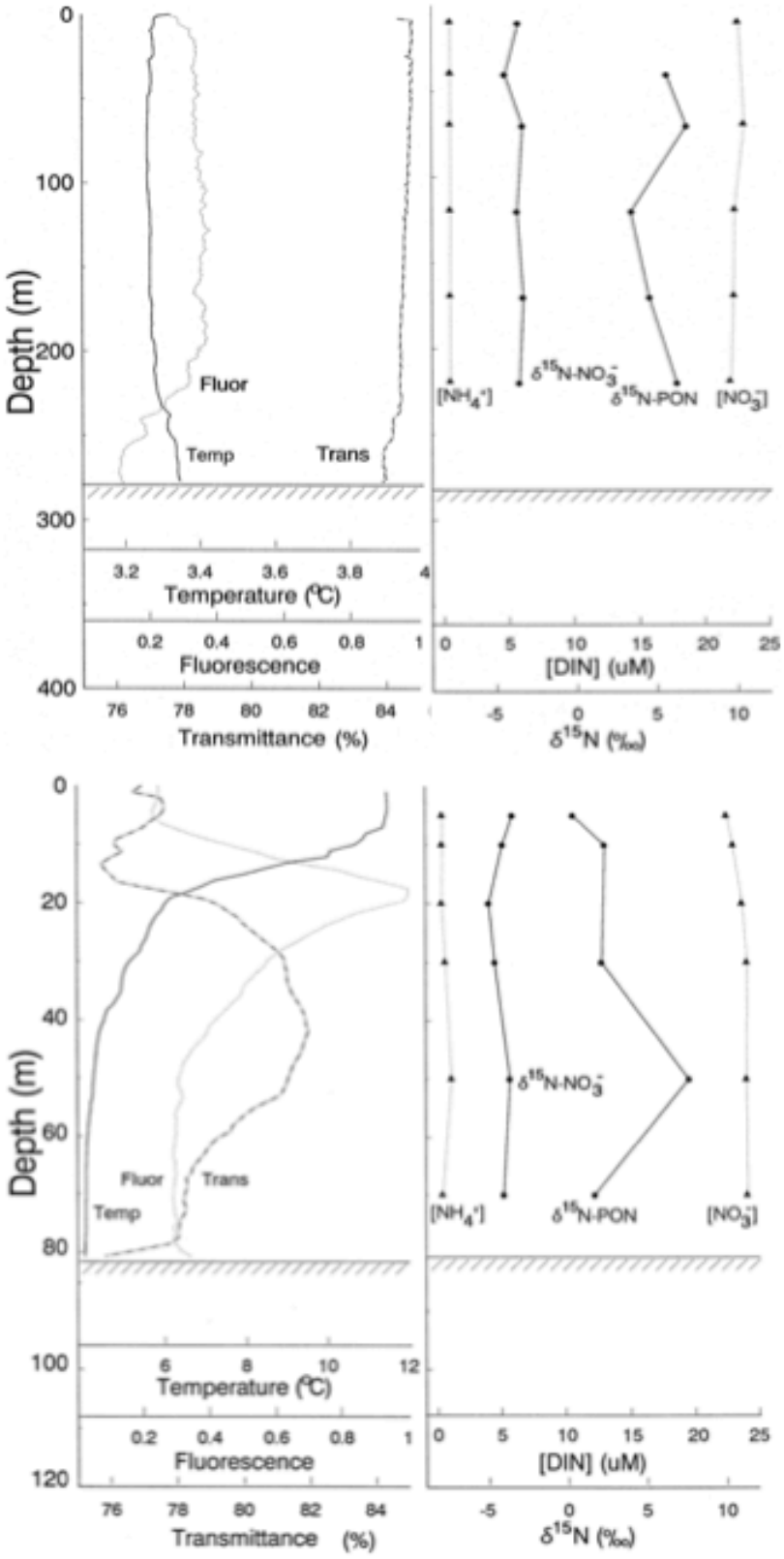
Depth (m)
Fluor
Temp
Trans
Temperature (°C)
Fluorescence
Transmittance (%)
[NH4+]
δ15N-NO3-
δ15N-PON
[NO3-]
[DIN] (uM)
δ15N (‰)

Fig. 11. Nitrate, dissolved inorganic nitrogen, and ammonia profiles for the central basin (top, mixed water column) and the western arm (bottom panel, stratified) of Lake Superior. Modified from Ostrom et al., 1998.

Interestingly, the abundance of total *Archaea* explained the variations of the *amo*A distribution better than the MG-I (r^2=0.77, p<0.001), which is not the case for data from Lake Malawi (see Chapter II). The abundance of MG-I correlated well with that of the total *Archaea*, suggesting that the Thaumarchaeota may be a principal component of the bacterioplankton in Lake Superior. This finding agrees with what has been reported previously (Small et al., 2013). Surprisingly, MG-I were detected in all the surface samples, which was not the case for Lake Malawi.

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Appendix 1
DAPI counts for available samples. Refer to Table 2 and 4 for sampling details.

Lake	Date	Site	Depth	Prokariotic cells/L x 10^8
Superior	September 2010	EM	5	17.8
			100	6.97
		ED	5	18.8
			100	7.67
			5	14.6
		CM	100	7.42
			5	16.0
		IR	100	6.94
			5	12.3
		WM	100	8.10
			5	14.0
		FW	100	6.05
Malawi	January 2011	Tchambo	10	15.5
			40	13.6
			70	8.11
			100	5.41
			140	3.29
			200	3.49
			230	7.48
			500	5.60
	November 2011	Senga Bay	10	23.1
			30	14.8
			50	18.1
			100	8.92
			150	1.63
Kivu	January 2012	K10	3	12.1
			20	19.9
		K11	60	8.91
			100	6.02